FEB 6 1935

VOL. 20 JANUARY, 1935 No. 4

MEDICAL LIBRARY

The Journal of Laboratory and Clinical Medicine

WARREN T. VAUGHAN, M.D., EDITOR
808 Professional Building
Richmond, Va.

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
University of Cincinnati, Cincinnati

Physiology
J. J. R. MACLEOD, M.B.
Physiological Laboratory, Marischal College, Aberdeen, Scotland

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Clinical Pathology
W. C. MACCARTY, M.D.
Mayo Clinic, Rochester, Minn.

T. B. MAGATH, M.D.
Mayo Clinic, Rochester, Minn.

Biochemistry
VICTOR C. MYERS, PH.D.
Western Reserve University, Cleveland

Experimental Medicine
RUSSELL L. HADEN, M.D.
Cleveland Clinic, Cleveland

Immunology
JOHN A. KOLMER, M.D.
Temple University, Philadelphia

ROBERT A. KILDUFFE, M.D.
Atlantic City, N. J.

Internal Medicine
GEORGE HERRMANN, M.D.
University of Texas, Galveston

Bacteriology
M. H. SOULE, Sc.D.
University of Michigan, Ann Arbor

Metabolism
CLIFFORD J. BARBORKA, M.D.
Northwestern University, Chicago

Surgery
DEAN LEWIS, M.D.
Johns Hopkins University, Baltimore

PUBLISHED BY THE C. V. MOSBY COMPANY, 3523-25 PINE BLVD., ST. LOUIS

Entered at the Post Office at St. Louis, Mo., as Second Class Matter.
Copyright 1935, by The C. V. Mosby Company

ELI LILLY AND COMPANY

FOUNDED 1876

Makers of Medicinal Products

For patients who prefer an inhalant without aromatics, Eli Lilly and Company suggest

Inhalant Ephedrine (Plain) With Merthiolate (No. 31)

A bland preparation with bactericidal and decongestive properties, indicated for the relief of inflammatory and infectious conditions involving the nasopharynx. It may be applied with excellent results in the form of a spray, by drops, or on cotton packs.

Prompt Attention Given to Professional Inquiries

PRINCIPAL OFFICES AND LABORATORIES, INDIANAPOLIS, INDIANA, U. S. A.

The Journal of Laboratory and Clinical Medicine

VOL. 20 JANUARY, 1935 NO. 4

CLINICAL AND EXPERIMENTAL

METEOROLOGICALLY CONDITIONED VARIABILITY OF SEROLOGIC TESTS IN SYPHILIS*

EMIL T. HOVERSON, M.A., M.D., KANKAKEE, ILL., AND
WM. F. PETERSEN, M.D., CHICAGO, ILL.
WITH THE TECHNICAL ASSISTANCE OF
MR. DELESTER SACKETT, ELGIN, ILL.

SINCE the introduction of the Wassermann test, the serologic diagnosis of syphilis has become a field that has attracted the interest of many workers, and as a result of this there is a voluminous literature on the subject. One point which this mass of work has served to have emphasized is the fact that a certain degree of uniformity obtains in all of the various tests for syphilis, so that despite the many modifications of the original Wassermann reaction, and even subsequent variations, there is a close agreement between the serologic findings obtained by the various methods (technical proceedings). Because of this close agreement, it is generally accepted that the results of a blood serum test for syphilis is quite accurate, regardless of the method employed for the determination. Moreover, if the testing is done by well-trained technicians, it is very unlikely that errors in diagnosis will occur except to a very limited extent. As these statements constitute common knowledge it is felt that they call for no further substantiation.

Another belief which is quite generally held is that a change in the serology from positive to negative or vice versa indicates some alteration in the concentration of the syphilitic antibodies. Moreover, since syphilis is regarded as a

*From the Kankakee State Hospital, and the University of Illinois, The State Psychopathic Laboratory, Department of Pathology, College of Medicine.
Received for publication, April 16, 1934.

disease process of a chronic progressive character, changes in the serology ought to be gradual, and quite uncommon in the absence of specific treatment. In those cases in which treatment is instituted, the changes in the serology are gradual, sometimes a period of years being required for a reversal from positive to negative findings. One of us (E. T. H.)[1] has elsewhere reported the occurrence of such similar changes in untreated syphilis, but in these instances lengthy periods of time had elapsed between the recording of the first positive test for syphilis, and the final negative test.

The purpose of the present study was to determine if reversals of the serology occurred over long or short periods of time. The results of the study indicate that in certain cases there occurred marked changes in the serology over short periods of time, measured in terms of days. As far as it has been possible to determine, no similar study has appeared in the available literature. True, there are many reports of a single series of cases, but in these the object has been a comparison between methods to establish correlation between different methods. Also comparisons have been made over lengthy periods, and following antisyphilitic treatment.

As is well known, definite variations are found in the blood serology in certain cases of syphilis as a sequel to treatment. Thus in a group of patients having strongly positive serologic evidence of syphilis before treatment, some will show a lessening of the strength of the reaction, and it will be recorded in accordance with common usage as three-plus, two-plus, one-plus, and negative. If the group showing these changes is given a rest period, a certain percentage will have a four-plus reaction, while some will be negative, after some months. These observations are common, but they have been made usually on single and not on consecutive blood samples.

Since, as was already stated, the methods of determination are standardized, and carried out by trained technicians, the results are quite accurate, and it follows that if changes are observed, they are due to other factors than those inherent in the determination itself. All the determinations in this work were made using the Wassermann and Kahn tests (Craig modification) on the same sample of blood. Routine qualitative determinations such as are usually made for ordinary work, were carried out on consecutive samples for a definite length of time. All of this technical work was done by Mr. DeLester Sackett. Because of this, all samples were handled in a uniform manner. The same batch of reagents, i.e., antigen, for every test was used, so that changes would be caused by the specimen and not the reagent.

For the purpose of this study, white male patients afflicted with general paralysis in varying stages of the disease were selected. The only requirements were, first, that the patient had showed positive serologic evidence of syphilis on admission to the Kankakee State Hospital; second, that he had received no form of treatment for at least six months prior to this work; third, that the superficial veins were accessible, and last, but quite important, that the patient himself gave cooperation.

The samples of blood were withdrawn in a uniform manner, on every Tuesday, Thursday, and Sunday during the course of the study, between the

hours of 8:30 and 9:30 A.M. The serum was collected on the same day on which the blood was withdrawn, and the next day it was sent to the State Psychopathic Laboratory.

The ages of the patients ranged from thirty to sixty years. All were free from other physical conditions which might affect the serology. Originally ten patients were selected, but as five of these had negative Wassermann and Kahn reactions on the blood sera on each of the first seven determinations, they were excluded from further participation in this study. In their place five other patients were chosen on the basis on which the first ten patients were selected.

In order to demonstrate the results, the fifteen patients are grouped in three classes as follows:

1. Five patients on whom both the blood Wassermann and Kahn reactions were negative.

2. Seven patients who showed rather striking variations in both the Wassermann and Kahn reactions.

3. Three patients who had strongly positive Wassermann and Kahn reactions on all determinations.

GROUP I

TABLE I

THIS TABLE INCLUDES BOTH THE WASSERMANN AND KAHN REACTIONS

DATE, 1934	PATIENT IDENTIFICATION NUMBERS				
	I A.B.	II S.L.	III G.B.	IV J.R.	V M.F.
January					
14	Neg.	Neg.	Neg.	Neg.	Neg.
15					
16	Neg.	Neg.	Neg.	Neg.	Neg.
17					
18	Neg.	Neg.	Neg.	Neg.	Neg.
19					
20					
21	Neg.	Neg.	Neg.	Neg.	Neg.
22					
23	Neg.	Neg.	Neg.	Neg.	Neg.
24					
25	Neg.	Neg.	Neg.	Neg.	Neg.
26					
27					
28	Neg.	Neg.	Neg.	Neg.	Neg.

Thus the five patients in this group had consistently negative Wassermann and Kahn reactions on their blood sera in seven determinations, taken in a period of two weeks.

GROUP II

In order better to show the variations, the results on the seven patients comprising this group are placed in two tables; the first (Table II) shows the variations in the Wassermann reaction, and the second (Table III), the changes in the Kahn reaction on the same seven patients.

It is believed that the changes shown in Tables II and III of Group II are significant, and are worthy of further study. The extent of the changes themselves is evident, and needs no comment.

TABLE II

WASSERMANN VARIATIONS

DATE, 1934	VI J.M.	VII M.K.	VIII F.W.	IX F.K.	X G.T.	XI H.E.	XII L.M.
January 14	−	−	+	−	+++		
15							
16	±	−	+	−	−		
17							
18	±	±	−	+	+++		
19							
20							
21	−	−	−	−	−		
22							
23	−	−	−	−	−		
24							
25	−	±	−	−	±		
26							
27							
28	++	−	−	−	−		
29							
30	+	++	++	−	++++	+	++
31							
February 1	+	+	+	−	++++	++++	−
2							
3							
4	−	−	−	−	−	−	−
5							
6	−	−	++	−	+	++++	−
7							
8	±	+++	++	−	±	++++	−
9							
10							
11	+++	−	−	−	++++	++++	−
12							
13	−	−	±	−	−	+	−
14							
15	+	++	++	−	+++	++++	+
16							
17							
18						++++	−
19							
20						++++	++
21							
22						++++	++
23							
24							
25						++++	−
26							
27						++++	+
28							
March 1						++++	−

GROUP III

The results in this group are placed in a single table (Table IV); the first three figures on the left show the Wassermann, and the remaining three columns show the Kahn variations.

The foregoing may be summarized by saying that there can be no doubt that all the patients in this study had on admission to the Kankakee State Hospital positive neurologic, mental, and laboratory findings of general paralysis, and hence all had at one time had syphilis. Further, all of the patients had been given some form of treatment, and in some, there resulted a reversal of the laboratory findings from positive to negative. In others the serologic find-

TABLE III

KAHN VARIATIONS

DATE, 1934	VI J.M.	VII M.K.	VIII F.W.	IX F.K.	X G.T.	XI H.E.	XII L.M.
January 14	±	++++	+++	−	++++		
15							
16	+	++++	++++	−	++++		
17							
18	+	+++	−	++++	++++		
19							
20							
21	−	−	++	−	++++		
22							
23	−	++	++	−	++++		
24							
25	±	++	+++	−	++++		
26							
27							
28	++	++++	+++	−	++++		
29							
30	+	++++	+++	−	++++	++++	+++
31							
February 1	++	++++	+++	−	++++	++++	+++
2							
3							
4	−	+++	+	−	++++	+	+++
5							
6	−	++	+++	−	++++	++++	+++
7							
8	++	++++	++++	−	++++	++++	++++
9							
10							
11	±	++	+++	−	++++	++++	++++
12							
13	−	++++	++++	−	++++	++++	++++
14							
15						++++	++++
16							
17							
18						++++	++
19							
20						++++	++
21							
22						++++	+++
23							
24							
25						++++	+++
26							
27						++++	++
28							
March 1						++++	++++

ings remained positive, and in still others there was noted a marked variation. The interest lies in the group comprising those patients on whom were noted variations in the serologic reactions. Apparently from the results, it is quite possible for a patient to have a four-plus Kahn or Wassermann on one day, and a few days later to have negative reactions.

Since it was believed that the variations were due to changes in the serum, and not due to laboratory error, an explanation was sought for in the organism itself. Since syphilis is a chronic progressive disease process, it does not seem reasonable to believe that the activity of the process underwent such rapid and marked changes. Hence it was thought that there were other factors which

underwent rapid changes, and in that way influenced the serology. On the basis of past work dealing with the effect of meteorologic changes on the organism, one of us (W. F. P.) has noted a close relationship between these changes, and the activity and physiologic functions of man. In order to establish the presence or absence of a correlation between the serologic variations and meteorologic changes, the meteorologic state has been compared with the serologic variations as follows: Thus, from January 13 to 17 there occurred a polar infall with a rising barometer. This episode is labeled 1 and 2 over the barograph. In Patient X the Wassermann reaction became positive on the fourteenth and eighteenth, i.e., immediately after the maximum of each meteorologic event. Patient IX became positive (Wassermann one-plus, Kahn four-plus) on the eighteenth. Patient VIII became positive to both the Kahn and Wassermann on the fourteenth and sixteenth. Patient VII had a positive Kahn on the seventeenth, and Patient VI became doubtful with Wassermann on the sixteenth and eighteenth, doubtful Kahn on the fourteenth, and one-plus Kahn on the sixteenth and eighteenth.

From January 23 to 26 two polar infalls occurred in rapid succession (labeled 3 and 4 on the barograph). In Patient X a doubtful Wassermann was recorded on the twenty-fifth. In Patient IX there was no alteration. In Patient VIII the Wassermann was unaltered, but the Kahn was increased on the twenty-fifth. In Patient VII the Wassermann became doubtful on the twenty-fifth

TABLE IV

DATE, 1934		WASSERMANN XIII J.B.	WASSERMANN XIV L.C.	WASSERMANN XV J.W.	KAHN XIII J.B.	KAHN XIV L.C.	KAHN XV J.W.
January	30	++++	++++	++++	++++	++++	++++
	31						
February	1	++++	++++	++++	++++	+++	++++
	2						
	3						
	4	+++	++++	++++	++++	+++	++++
	5						
	6	++++	++++	++++	++++	++++	++++
	7						
	8	++++	++++	++++	++++	++++	++++
	9						
	10						
	11	++++	++++	++++	++++	++++	++++
	12						
	13	++++	++++	++++	++++	++++	++++
	14						
	15	++++	++++	++++	++++	++++	++++
	16						
	17						
	18	++++	++++	++++	++++	++++	++++
	19						
	20	++++	++++	++++	++++	++++	++++
	21						
	22	++++	++++	++++	++++	+++	++++
	23						
	24						
	25	++++	++++	++++	++++	+++	++++
	26						
	27	++++	++++	++++	++++	++++	++++
	28						
March	1	++++	++++	++++	++++	++++	++++

and the Kahn became positive on the twenty-third and twenty-fifth, and in Patient VI the Kahn became doubtful on the twenty-fifth.

A major polar infall began on the twenty-eighth[5] and reached maximal extent on the twenty-ninth and thirtieth of January with zero weather. Patient

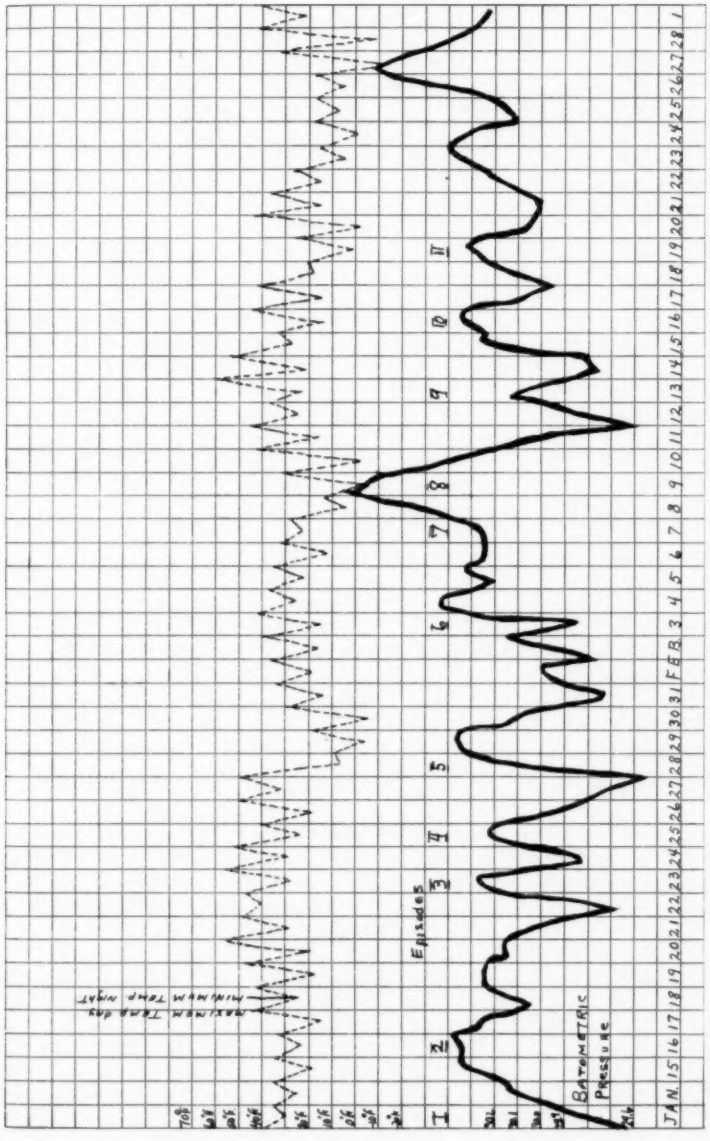


Chart 1.

XI had a two-plus Wassermann on the thirtieth, Patient X had a four-plus Wassermann on the thirtieth, and on February 1. Patient IX was unaltered, Patient VIII had a two-plus Wassermann on the thirtieth and a one-plus on the first. Patient VII had a four-plus Kahn on the twenty-eighth, and a two-plus on the thirtieth, and a four-plus on the first. He had a two-plus Wassermann on the thirtieth, and a one-plus on the first. Patient VI had a two-plus Was-

sermann and Kahn on the twenty-eighth, one-plus Wassermann on the thirtieth and first, and a one-plus Kahn on the twenty-eighth, and a two-plus on the first.

The next great disturbance followed in the form of three polar infalls that culminate in unusually high barometric pressure. These episodes have been labeled 6, 7, and 8. The reaction in Patient XI was unaltered, Patient X had a one-plus Wassermann and a doubtful Wassermann on the sixth and eighth. The reaction in Patient IX was unaltered, and Patient VIII developed a two-plus Wassermann and a four-plus Kahn. Patient VII had a three-plus Wassermann on the eighth, and a four-plus Kahn at the same time. Patient VI developed a doubtful Wassermann and a two-plus Kahn on the eighth. Probably as a delayed reaction to the stimulus we found the positive Wassermanns of Patients X, IX, and VI on the eleventh.

A series of less intense meteorologic changes now followed with polar infalls numbered 9, 10, and 11. In Patient XI the effects could be readily followed because the observations were continued and each polar infall was associated with a change in the serologic reaction.

It is not our purpose at this time to enter into a discussion of the mechanisms here involved, since the study is part of related observations in other field;[2] we wish merely to call attention to the fact that even such seemingly certain serologic reactions as those of syphilis are subject to the constant biologic tide as it is conditioned by the meteorologic environment. In later studies we shall present further details.

Chart I shows in detail the facts just discussed. The chart shows the changes in the barometric pressure, and external temperature during the period from January 13 to March 1, 1934, inclusive. The dotted line indicates the various temperature changes. This includes the maximum and minimum temperature readings. It so happens that the maximum readings occurred during the day, and the minimum occurred during the night. The heavy shaded line shows the changes in the barometric pressures. The figures on the left hand side indicate the readings in inches of mercury. Above the barograph, are number episodes which have been compared with the serologic variations.

CONCLUSIONS

1. A single blood Wassermann or Kahn test, if negative, means little or nothing as far as ruling out syphilis is concerned.

2. There occurs in some individuals having syphilis rather striking variations in the serology, and these variations take place in terms of days.

3. In those cases where variations occur, there seems to be no absolute agreement between the Wassermann and Kahn tests.

4. It is believed that the variations in the serology are conditioned by meteorologic changes.

REFERENCES

1. Hoverson, Emil T.: The Reversal of the Blood Wassermann Reaction in Untreated Syphilis, Am. J. Syph. 17: 392, 1933.
2. Petersen, Wm. F.: The Patient and the Weather 3: Ann Arbor, 1934, Edwards Bros.

CREATININE CLEARANCE DURING THE HYPERTHERMIA OF DIATHERMY AND FEVERS*

WILLIAM H. GRANT, M.D., AND GRACE MEDES, PH.D., MINNEAPOLIS, MINN.

ALTHOUGH Bieter (1933) observed that rate of blood flow to the glomerulus and rate of glomerular filtration in a frog's kidney perfused by Richard's method, were increased by heating the afferent artery, the effects of general systemic hyperthermia have been a subject of some disagreement. McIntosh and Reimann (1926) found that out of 13 cases of pneumonia, 9 patients showed renal hyperfunction as demonstrated by the phenolsulphonephthalein test and the urea concentration index, while 4 showed a moderate depression. In a review of the literature dealing with renal function in pneumonia, these authors found that out of 6 investigators, 3 reported impairment of kidney function, 2 reported absence of function and one emphasized the occurrence of renal hyperfunction. Goldring (1931) in acute rheumatic fever, found urea clearances more elevated than the highest normal values. Ambard (1920) lowered the temperatures of dogs by wrapping their shaved abdomens in ice and found the uremic debit and the ureo-secretory constant lowered.

In view of the discrepancies of these findings, we have studied (A) creatinine clearances in dogs during the hyperthermia produced by diathermy and (B) creatinine clearances in patients with fever caused by infection to determine (A) the effect of general systemic hyperthermia on creatinine clearance and (B) the extent to which any changes observed in infectious fevers may be referred to the elevation of body temperature.

EXPERIMENTS

A. Hyperthermia of Diathermy.—For the diathermy experiments male dogs varying from 13 to 21 kg. body weight were employed. No anesthetic was used, the dogs having been previously trained to lie quietly throughout the procedure, strapped to a dog board in a comfortable position. The technic of the test was the same as that described by Rehberg (1931) for clinical use, except that 2 gm. of creatinine dissolved in 10 to 15 c.c. of warm saline solution were injected into the saphenous vein before starting. The dogs were then given 250 c.c. of warm water by stomach tube to ensure adequate diuresis. All urine was taken by catheter and the bladder washed repeatedly with warm saline solution until the washings were clear.

The type of diathermy apparatus employed was described by Hemingway and Stenstrom (1932). The electrodes which consisted of two copper-mesh plates attached to thick pieces of cotton padding, were soaked in 1 per cent saline

*From the Department of Medicine, University Hospital.
Received for publication, May 3, 1934.

solution and applied to the shaved sides of the thorax and abdomen of the dogs. These pad-electrodes were kept moist with saline solution throughout the experimental period.

At the beginning of the test the dog's rectal temperature was taken. The electrodes were then connected to the diathermy machine and the current turned on. Medium voltage and a current ranging between 1,000 and 2,000 ma. were used. The dog was covered with a blanket to prevent excessive heat loss and when the temperature was elevated from 0.8° to 1.5° C., a process requiring upward of ninety minutes, the test was begun. Four dogs were employed and 6 to 8 experiments made on each dog, 3 or 4 at normal temperature (control periods) and a similar series using diathermy. Rest periods of several days were allowed between tests.

B. Hyperthermia in Fevers of Infectious Origin.—Patients with hyperthermia (any temperature above 37.2° C.) were tested by Rehberg's method, both during the febrile period and after their temperatures had become normal.

RESULTS

Filtration and Temperature.—The results of the diathermy experiments are given in Table I. There was in each case an increase in the creatinine clearance during diathermy, but no correlation between the average number of degrees the temperature of the different dogs was raised and the increase in their

TABLE I

RESULTS OF DIATHERMY EXPERIMENTS ON 4 DOGS, GIVING GLOMERULAR FILTRATIONS (CREATININE CLEARANCES) WITH RECTAL TEMPERATURES (1) BEFORE AND (2) DURING THE TESTS. EACH FIGURE IN THE TABLE REPRESENTS THE AVERAGE OF THE FINDINGS OF 3 OR 4 SEPARATE EXPERIMENTS WITH INTERVALS OF ABOUT 3 DAYS BETWEEN TESTS

DOG	BODY WEIGHT KG.	NO. TESTS	DIATHERMY	TEMPERATURE 1 ° C.	TEMPERATURE 2 ° C.	FILTRATION C.C./MIN.
1	16.0	3	Without	39.1	39.1	78.7
		3	With	39.2	39.8	94.5
2	13.2	3	Without	38.3	38.3	36.6
		4	With	38.6	39.6	78.6
3	20.9	4	Without	39.2	39.2	79.2
		4	With	39.1	40.1	94.0
4	15.5	4	Without	38.6	38.7	60.0
		4	With	38.9	40.0	75.5

creatinine clearances. Thus, Dog 1, with an average elevation of temperature of 0.6° C. had an average increase in filtration of 15.8 c.c. per minute, while Dog 2, with an average rise in temperature of 1° C. had an increase in filtration averaging 42 c.c. per minute. There was, however, a correlation between the two factors in each individual dog. For instance, three tests with diathermy were made on Dog 1, with increase in temperature of 0.3°, 0.7° and 1.1° C., respectively, while the corresponding filtrations increased 8.8, 13.1, and 25.6 c.c. per minute. These findings are similar to those of Medes and Herrick (1933) for the relation between blood flow to the kidney and creatinine clearance, where a general parallelism was found to exist between the two phenomena in each individual dog, although the same close relationship did not hold between the clearances and blood flow in different dogs.

The results of the experiments with fever due to infection are given in Table II. There was no indication of a general increase or decrease in kidney function when the temperature was moderately elevated. However, in every case studied in which the temperature was raised to 38.9° C. or above there was increased filtration. In those cases (Cases 4, 6, 7, 11, 13) in which there was the greatest increase in kidney function during hyperthermia, the elevation in temperature ranged from 1.0° to 2.3°. In the three cases of pneumonia (Cases 1, 14, 15) the filtration was increased during the period of fever.

TABLE II

TEMPERATURES, BLOOD PRESSURES AND GLOMERULAR FILTRATIONS (CREATININE CLEARANCES) OF 15 PATIENTS (1) DURING AND (2) FOLLOWING FEVERS OF INFECTIOUS ORIGIN

PATIENT	CLINICAL DIAGNOSIS	AGE YR.	TIME BET. TESTS DAYS	TEMPERATURE ° C. 1	2	BLOOD PRESSURE MM. HG 1	2	FILTRATION C.C./MIN. 1	2
1	Bronchopneumonia	47	6	38.3	36.9	100/ 68	96/ 66	146.9	90.4
2	Agranulocytic angina	56	13	39.0	36.7	116/ 64	96/ 60	262.2	73.1
3	Pyelonephritis, cystitis	22	10	38.9	36.6	140/100	108/ 76	187.4	124.5
4	Neurosyphilis, malarial fever	56	12	38.6	36.9	110/ 65	104/ 60	186.5	142.3
5	Cardiorenal nephritis	45	6	37.8	36.9	170/140	162/128	63.3	77.4
6	Sexual neurasthenia	22	6	38.2	37.0	136/ 84	124/ 80	176.6	102.1
7	Hypertension, cardiac decompensation	56	6	38.1	37.1	164/ 84	154/ 90	132.8	19.6
8	Pleurisy with effusion	49	6	38.7	37.0	134/ 86	120/ 74	67.9	110.2
9	Polyarthritis	20	10	38.3	36.6	100/ 80	115/ 80	120.8	166.4
10	Diphtheria	27	7	38.1	36.3	124/ 80	110/ 80	155.8	173.6
11	Hodgkin's disease	60	22	38.3	36.9	104/ 60	96/ 58	236.6	107.5
12	Chronic nephritis, dermatitis	29	7	37.9	37.0	126/ 64	124/ 64	69.0	77.4
13	Chronic cholecystitis	31	8	37.9	36.7	130/ 80	102/ 68	144.2	92.5
14	Lobar pneumonia type III	24	13	40.5	36.8	130/ 72	120/ 70	545.2	174.0
15	Hypertension	32	15	38.9	36.9	132/ 96	126/ 92	222.6	132.1

As in the diathermy experiments, the differences in the filtrations during hyperthermia and after the temperatures had subsided to normal, bore no definite relation to the differences in temperature. For instance, Case 4, with a difference in the temperature of 1.7°, showed only a decrease of 44 c.c. per minute in the filtration, while Case 7, with a difference of but 1°, decreased 113 c.c. per minute in filtration.

Filtration and Body Weight.—It has previously been shown by Medes and Berglund that there is no correlation between body weight and rate of glomerular filtration in adult human beings under the conditions of Rehberg's test. That such a general relationship holds for large groups, however, may be seen by comparing the filtration per kilo body weight of man and dogs at approximately the same body temperature. For this purpose the filtration rates of dogs at normal temperatures (range from 38° to 39.4°, average 38.8°) may be compared with the rates of patients with infectious fevers (range from 37.8° to 40.5°, average 38.7°). The average filtration rate per kilo of body weight for the dogs was 3.9 c.c. per minute and for the patients 3.1 c.c. per minute. The same patients at normal temperature (36.8°) had an average filtration rate of 1.8 c.c. per minute per kilo body weight, and the dogs during diathermy (average temperature, 39.9°) had filtration rates of 5.2 c.c. per minute. Medes and

Berglund, in the study mentioned above, found an average filtration rate per kilo body weight of 2.4 in sixty normal medical students. Had a group with this filtration rate been subjected to a rise of temperature similar to that of the patients, it seems probable that a value more nearly approaching the 3.9 found on dogs would have been obtained.

The increase in the rate of filtration for each degree rise in body temperature varied considerably and showed no constant relation to body weight. Thus, Dog 2, which weighed 13.2 kg., had an average increase of filtration per degree rise in temperature of 42 c.c. per minute, whereas the heaviest dog (weighing 20.9 kg.) had a rise in its creatinine clearance of only 14.8 c.c. per minute per degree elevation of body temperature, and for Dog 4, weighing 15.5 kg., the corresponding increase in filtration was 14.1 c.c. per minute. The average increase in filtration for each degree rise in temperature found in sixteen experiments on the dogs was 10 c.c. per minute, a rise of 28 per cent. The corresponding average increase in the patients was 25.7 c.c. per minute, an increase of 23 per cent.

The striking agreement in filtration rates per unit body weight at approximately the same temperature in dogs and man, together with the agreement in the percentage increase in filtration per degree rise in temperature, indicates that in measurements of filtration we are dealing with a process fundamentally related to body metabolism.

Filtration and Blood Pressure.—Medes and Bellis (1933) showed a direct relationship between blood pressure in the renal artery and glomerular filtration in dogs, as measured by the creatinine clearance test. Nine of our cases of fever due to infection in which there was some increased filtration during fever also showed some increase in the blood pressure during this period (Cases 1, 2, 3, 6, 7, 11, 13, 14, 15). Patient 5 who showed renal impairment during both tests, had a slightly lower clearance during hyperthermia than after, while the blood pressure was slightly higher during hyperthermia. Case 9 showed both a decreased filtration and blood pressure during hyperthermia. Since this case was in a single class a short history is given: female, aged twenty, entered the hospital two weeks postpartum suffering with an acute polyarthritis. The usual kidney function tests were within normal limits. The Rehberg test was also normal, showing a filtration of 120.9 c.c. per minute. The temperature at the time of the test was 38.3° while the blood pressure was 100/80. Ten days later when the temperature had dropped to 36.6° the test was repeated. At this time the filtration was found to be 166.4 c.c. per minute, while the blood pressure was 115/80. The findings in this case conform to the work of Janney and Walker (1932) who concluded from a water diuresis test that there is generally a decreased kidney function at the time of delivery, and that this kidney function gradually increases postpartum until it again reaches its normal value.

Ellis and Weiss (1933) studied 18 cases of arterial hypertension by the Rehberg test. Out of this series 13 showed normal results, two showed slight reduction in function, while three showed marked reduction. No mention of temperature was given in their report. Two of the cases studied in our series had hypertension (Cases 5 and 7). Case 5 remained below normal during both

tests, while Case 7 dropped from a clearance well within normal limits during hyperthermia to one markedly below normal after the hyperthermia had disappeared.

CONCLUSIONS

In contrast to the general parallelism that exists between body temperature and creatinine clearance in dogs during diathermy when all other factors are being held as nearly constant as possible, the creatinine clearances in patients with fever undergo wide variations. In the series of 15 patients observed here, the clearances during the height of the fever varied from 600 to 60 per cent of their postfebrile values.

A survey of the relationship between blood pressure and filtration during the fever, revealed similar irregularities: nine patients showed elevation of all three factors, temperature, blood pressure, and filtration; three showed increase in blood pressure with fall in filtration; in one patient filtration and blood pressure were lowered, while in the remaining two cases the changes in blood pressure and filtration were insignificant.

The group whose behavior is the most difficult to explain on a theoretical basis is that in which blood pressure and temperature were both elevated while filtration was lowered. Two other known factors may be considered as responsible for this behavior: (a) there may be a decrease in the volume flow of blood to the kidney in spite of elevation of temperature and blood pressure, and (b) there may be a direct effect of the infection on the kidney, resulting in a temporary closing off of some of the glomeruli or in an altering of their permeability.

The authors wish to express their thanks to Dr. Allan Hemingway of the Department of Physiology for the use of his diathermy apparatus.

REFERENCES

Ambard, L.: Physiologie normale et pathologique des reins, Paris, 1920.
Bieter, R. N.: Personal communication to the authors.
Ellis, L. B., and Weiss, S.: Renal Function in Arterial Hypertension, J. A. M. A. **100**: 875, 1933.
Goldring, W.: Studies of the Kidney in Acute Infection. II. Observations With the Urea Clearance Test in Acute Rheumatic Infection, J. Clin. Investigation **10**: 345, 1931.
Hemingway, Allan, and Stenstrom, W. K.: Physical Characteristics of High Frequency Currents, J. A. M. A. **98**: 1446, 1933.
Holten, C., and Rehberg, P. B.: Studies on Pathological Function of Kidneys in Renal Disease, Especially Bright's Disease, Acta med. Scandinav. **74**: 479 and 538, 1931.
Janney, J. C., and Walker, E. W.: Kidney Function in Pregnancy. I. Water Diuresis in Normal Pregnancy, J. A. M. A. **99**: 2078, 1932.
McIntosh, J. F., and Reimann, H. A.: Kidney Function in Pneumonia, J. Clin. Investigation **3**: 123, 1926.
Medes, Grace, and Bellis Carroll, J.: The Effect of Altering Renal Blood Pressure on Glomerular Filtration, Am. J. Physiol. **107**: 227, 1933.
Medes, Grace, and Berglund, Hilding: Studies in Rehberg's Creatinine Test for Glomerular Filtration, in the Kidney in Health and Disease, Philadelphia, Lea and Febiger. (In press.)
Medes, Grace, and Herrick, J. F.: Blood Flow to the Kidney and Creatinine Clearance, Proc. Soc. Exper. Biol. & Med. **31**: 116, 1933.

THE EFFECT OF ALCOHOL UPON SPLEEN VOLUME*

F. D. McCrea, Ph.D., D. F. Marion, B.S., W. W. Tyson, and W. P. Kavanagh, A.B., Durham, N. C.

MOST investigators agree that small quantities of alcohol cause dilatation in the cutaneous area and constriction in the splanchnic area, the latter to a somewhat lesser degree. The blood pressure may either show no change or be slightly increased or diminished (Dixon,[1] Brooks,[2] Stewart,[3] Lieb,[4] Hyatt,[5] McDowall[6]).

Few references concerning the action of ethyl alcohol upon spleen volume appear in the literature. Barcroft and Rothschild[7] reported that alcohol administered by stomach to unanesthetized dogs had little effect upon the spleen until large doses (8 c.c. of alcohol per kilogram) were given, when the spleen gradually decreased in size, the maximum diminution, amounting to 42 per cent, occurring some twenty minutes later. Definite signs of alcoholic intoxication were noted. In cases where no constriction was noted, the animals were apparently not intoxicated by the dose used. Karitsky[8] states that while alcohol paralyzes the central nervous system, it almost never abolishes splenic contractions.

The following report presents further data concerning the action of small quantities of ethyl alcohol upon the motility of the spleen.

Method.—Cats were used. They were anesthetized with urethane intraperitoneally and prepared to register blood pressure and spleen volume as in preceding work.[9] Alcohol 1 c.c. in concentrations varying from 2 to 20 per cent was administered via the femoral vein.

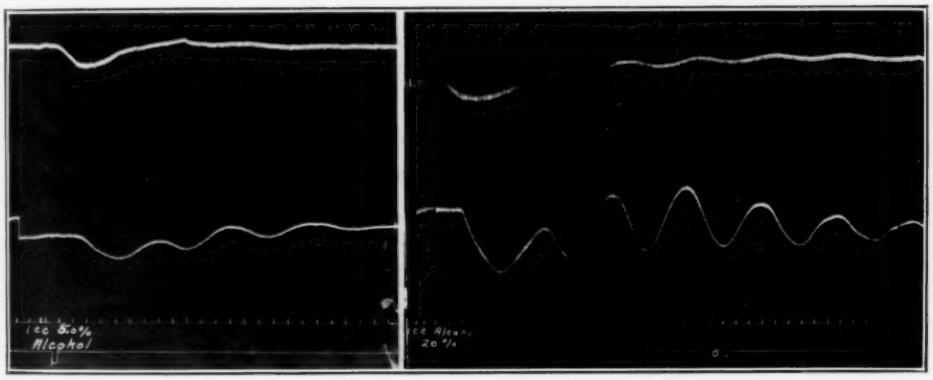
Results.—With threshold or slightly higher doses of alcohol, systemic blood pressure always fell slightly and quickly recovered, while spleen volume invariably diminished, its degree of change varying more or less directly with the concentration of the alcohol. Rhythmical movements of the spleen also occurred after the initial contraction, resembling those obtained with other drugs (Schaffer and Moore[10] and Barcroft and Nisimaru[11]). These results were noted in a total of 34 experiments with 8 cats. There seemed to be less relationship between the concentration of alcohol and the blood pressure, since in only a few cases was there an appreciably greater drop with 20 per cent than with 5 per cent alcohol. With larger doses (2 to 4 c.c. of 15 or 20 per cent) there was in most cases a greater fall and less prompt recovery.

In Fig. 1, *A* is seen the result of a dose of 1 c.c. of 5 per cent alcohol. Fig. 1, *B* shows the action of 1 c.c. of 20 per cent alcohol. The vascular depression is approximately equal in each and recovery almost equally prompt. How-

*From the Department of Physiology and Pharmacology, Duke University Medical School.
Received for publication, May 19, 1934.

ever, the degree of contraction of the spleen is much increased by the higher concentration of alcohol. Furthermore the rhythmical contraction and relaxation is much more pronounced and the spleen has a tendency to retain a higher tonus than that obtaining before alcohol, or than with concentrations less than 10 per cent, in which return to the control volume was almost always complete.

Discussion.—Henning[12] reported that abolition of the influence of the central nervous system by urethane narcosis stops the contraction of the spleen in mice and rats. Urethane has been the anesthetic routinely used in investigating the motility of the spleen in cats over a period of several years. At no time has urethane, when administered in sufficient quantity to secure surgical anesthesia, altered the splenic response to any very evident extent. Deep anesthesia undoubtedly does reduce the celerity and magnitude of its response, a point noted by De Boer and Carroll[13] and when combined with ether causes also some reduction of systemic blood pressure, as Barcroft[14] has pointed out. The ideal



A B.

Fig. 1.—A, Effect of 1 c.c. of 5 per cent ethyl alcohol. B, Effect of 1 c.c. of 20 per cent ethyl alcohol. Tracings from top to bottom, carotid pressure, spleen volume, time six second intervals and signal markers denoting injection.

method of ascertaining the effects of drugs undoubtedly necessitates an animal which is not anesthetized. However, so far as the splenic reaction to various drugs has been studied on anesthetized and unanesthetized animals the response is in the same direction according to most investigators. Contrary to Barcroft and Rothschild[7] we find that small intravenous doses of alcohol produce considerable contraction of the spleen, although with large doses our results are substantially the same as theirs. A possible explanation of this difference lies in the mode of administration, the effective stimulus probably being the concentration of alcohol in the blood. Brooks[2] demonstrated that alcohol introduced into the stomach by way of a gastric fistula caused no immediate vascular response, but a gradual reduction of systemic pressure occurred after some fifteen minutes. Likewise he found that slow intravenous injections of alcohol ranging from 0.5 to 4.0 c.c. per kilogram of animal were without effect other than cardiac acceleration and a gradual reduction of systemic pressure which invariably followed.

The rhythmical contractions produced in the spleen by alcohol, which are so pronounced in Fig. 1, *B*, occur in every record in which threshold or higher doses of alcohol are administered. These rhythmical waves are moreover seen to cause rhythmical fluctuations of the systemic blood pressure. The magnitude and duration of the splenic waves increased as the dose of alcohol used, occasionally not disappearing completely from the record for more than an hour after its administration.

Summary.—Small doses of dilute alcohol administered intravenously to cats anesthetized with urethane cause contraction of the spleen. This contraction is followed by rhythmical relaxation and contraction which may last for a considerable period of time.

REFERENCES

1. Dixon, W. E.: The Action of Alcohol on the Circulation, J. Physiol. **35**: 346, 1907.
2. Brooks, C.: The Action of Alcohol on the Normal Intact Unanesthetized Animal, J. A. M. A. **55**: 372, 1910.
3. Stewart, G. N.: Further Observations, Chiefly Pharmacological, on the Criteria by Which Deficiencies in the Blood Flow (in the Hands or Feet) Due to Mechanical Causes May Be Discriminated From Changes Due to Functional (Vasomotor) Changes, J. Pharmacol. & Exper. Therap. **8**: 281, 1915.
4. Lieb, C. C.: The Reflex Effects of Alcohol on the Circulation, J. A. M. A. **64**: 898, 1915.
5. Hyatt, E. G.: The Action of Alcohol on the Heart and Respiration, J. Lab. & Clin. Med. **5**: 56, 1920.
6. McDowall, R. J. S.: The Action of Alcohol on the Circulation, J. Pharmacol. & Exper. Therap. **25**: 289, 1925.
7. Barcroft, J., and Rothschild, P. W.: The Effect of Certain Anesthetics on the Volume of the Exteriorized Spleen, Arch. Internat. de Pharmacodyn. et Therap. **38**: 569, 1930.
8. Karitsky, B.: Über die Wirkung des Alkohols auf die Milzsperre, Arch. f. Gynäk. **88**: 146, 1931.
9. McCrea, F. D.: The Stimulant Action of Nicotine Upon the Spleen, J. Pharmacol. & Exper. Therap. **44**: 457, 1932.
10. Schaffer, E. A., and Moore, B.: On the Contractility and Innervation of the Spleen, J. Physiol. **20**: 1, 1896.
11. Barcroft, J., and Nisimaru, Y.: The Cause of the Rhythmical Contraction of the Spleen, J. Physiol. **74**: 299, 1932.
12. Henning, N.: Experimentelle Untersuchungen über die Milzsperre, Ztschr. Exper. Med. **54**: 317, 1927.
13. De Boer, S., and Carroll, D. C.: The Mechanism of the Splenic Reaction to General CO Poisoning, J. Physiol. **49**: 312, 1924-25.
14. Barcroft, J., and Nisimaru, Y.: Undulatory Changes of Blood Pressure, J. Physiol. **74**: 311, 1932.
15. Gradinescu, A., and Marcu, I.: L'Oncographie Splénique et Rénal et la diurèse sous L'Influence de la Éphédrine, Compt. rend. Soc. Biol. **96**: 27, 1927.

SOME STUDIES ON THE ETIOLOGY OF GRANULOMA INGUINALE*

HILDRUS A. POINDEXTER, M.D., WASHINGTON, D. C.

IN THE United States, with the exception of certain localized areas in some of the southern states, granuloma inguinale in the past has been referred to as a rare malady. Within more recent years, interest has been focused on this clinical condition primarily for three important reasons: First, the malady is becoming more prevalent and more generalized geographically; second, it is being considered by some as a fourth venereal disease; and third, there is a lack of agreement as to the nature of its etiology.

We have made microscopic examinations of nine clinical cases in Freedmen's Hospital within the last three years. Three of these patients lived within the vicinity of the District of Columbia for at least five years prior to the appearance of any lesion, and the other five came from various parts of the southern states.

Upon repeated smear examinations, seven of these nine cases showed the microscopic diagnostic picture of granuloma inguinale, when stained by the Wright or Giemsa method. The exact nature of these included bodies is not known.

Some observers agree with Donovan,[1] that it is a protozoan of the gregarine order of sporozoa; some with Flu,[2] that it is a chlamydozoan and there are others among whom are many of the more recent workers such as Campbell,[6] Randall, Small and Belk[7] who agree with Walker,[3] that it is a member of the Friedländer group of bacilli.

Five of these nine cases have been reported by Poindexter.[4] The other four cases were observed since. Three are still being treated at the Freedmen's Hospital, while one has been discharged as cured following tartar emetic and fuadin medication.

Within the last two years, attempts have been made to isolate and culture the organism in all cases that were positive by microscopic examination. Of the last four cases, three were positive microscopically. From the three positive cases of this set we were able to isolate identical organisms in two. By returning to the study of the first five cases, we were able to isolate a similar organism from one even though the patient had received considerable treatment and was improving nicely.

From the study of these three similar organisms, it is hoped that additional evidence may be shown as to the nature of the intracellular bodies (etiologic agent) in granuloma inguinale.

*From the Department of Bacteriology, Preventive Medicine and Public Health, College of Medicine, Howard University, and Freedmen's Hospital.
Received for publication, May 17, 1934.

The organisms which form the basis of this report were obtained from three clinical cases of the disease: Cultures from the first of these patients, M. N., one of those reported by Poindexter,[4] show the following organisms on isolation and study: (a) A gram-negative diplobacillus; (b) a small gram-positive staphylococcus and micrococcus; (c) a short chain nonhemolytic streptococcus; (d) a diphtheroid; (4) a few large thick rods which proved to be Döderlein's bacilli; and (f) the original smear from the patient also showed a spirochete which along with a fusiform bacillus gave a picture resembling that of a Vincent's angina smear. This fusiform bacillus is believed to be the same as the Döderlein's bacillus isolated by culture.

Cultures from the area of ulceration of the second patient, J. B., showed a pure culture of an organism similar in morphology and culture to the organism (a) isolated from the first patient, M. N. Cultures from the third patient, R. F., who is now on the ward receiving treatment with fuadin, contained the following organisms: (a) A small gram-negative bacillus which resembled Friedländer's bacillus morphologically. (b) A small gram-negative coccus, which corresponded culturally to that of *Micrococcus ureae* of Cohen; the gram-negative staining character was the exception, and (c) an organism of the diphtherial group.

The organisms (a) of the first and third patient, and the pure culture from the second were selected as the organisms of special study. The fact that three similar organisms appeared in three clinical cases of granuloma inguinale and that in one of these cases a pure culture was found, is significant from an etiologic standpoint. The selection of these organisms for study is in accordance with the opinion of Walker,[3] 1918, DeMonbreun and Goodpasture,[8] 1933, and others. Their thermal death point was 51° for thirty minutes, and 59° for ten minutes. The organisms did not show capsule formation in culture. Their biochemical reactions are shown in Table I.

EXPERIMENTAL

Fourteen mice and nine guinea pigs were used for the pathogenicity test of the organism in an attempt to fulfill Koch's postulates. Five mice were inoculated intraperitoneally with a suspension of the diplobacilli isolated from the first case; three of them with a saline suspension of a twenty-four-hour agar slant culture and the other two with ¼ c.c. of a twenty-four-hour broth culture. The mice showed no ill effects from the inoculation. After four days one of the mice was killed and cultures were made from the peritoneal cavity and the heart's blood. The peritoneal cavity showed considerable polymorphonuclear exudate and the culture was positive for the diplobacilli, but the culture from the heart's blood was negative.

Another one of the mice was sacrificed on the ninth day, with negative results. The other three mice continued to live without any ill effects for two months, after which they were used in a trypanosome experiment. Four mice were used in a similar way to test the pathogenicity of the diplobacilli isolated from the second case. The pathogenicity results were negative. Similarly five mice were used for intraperitoneal and sacrification tests with cultures from the

third patient with similar negative results. The guinea pigs were inoculated with doses twice the size of those given the mice. Three guinea pigs were used for each organism. The guinea pigs showed no reaction for eight days, after which time they appeared to be ill. Aseptic puncture of the peritoneal cavity resulted in the removal of some exudate similar to the type observed in mice, but the cultures for the organisms were negative. Similar punctures of the heart gave negative cultures. Within the following three weeks, the animals showed progressive loss of weight which resulted in marked emaciation and death about four weeks from the date of inoculation. Cultures taken by punctures from the peritoneum and heart were negative eight days after injection and remained negative as the emaciation progressed. By repeated experiments we were able, however, to obtain positive cultures of the organisms from fluid drawn from the peritoneum within the first six days after inoculation. The stools and urine were negative for the organism. At the autopsy there were no ulcerations or areas of granulation which in any way resembled those seen in granuloma

TABLE I

THE BIOCHEMICAL REACTIONS OF THE GRAM-NEGATIVE DIPLOBACILLI OBTAINED FROM THREE CASES OF GRANULOMA INGUINALE

MEDIA	FIRST PATIENT	SECOND PATIENT	THIRD PATIENT	OTHER CHANGES IN THE CULTURES
Dextrose	a*	a	a	Turbidity and a gray yellow fine granular precipitate.
Dextrine	–	–	–	A slight surface film and precipitate.
Maltose	a	a	a	A heavy yellow precipitate and a surface film.
Saccharose	a	a	a	A gray yellow precipitate and a surface film.
Lactose	a	a	a	A stringy granular flocculation.
Salicin	a	a ±	a	A thick gray film on the surface.
Mannite	a	a	a	Slight surface film and a gray yellow precipitate.
Inulin	–	–	–	Granular flocculation with a slight gray precipitate.
Inosite	–	–	–	A slight surface film and gray precipitate.
Sorbite	–	–	–	A slight surface film and slight precipitate.
Xylose	–	–	–	
Litmus milk	Sl.a*	Sl.a	a	No coagulation.
Plain agar	Grayish yellow slightly elevated. Glistening round colonies.			Similar reactions were observed for each of the other two organisms.
Peptone water	No indol formation.			Similar reactions were observed for each of the other two organisms.
Nutrient broth	Cloudy growth in 24 hours.			Similar reactions were observed for each of the other two organisms.
Gelatin	Not liquidified but bubbles of gas occur just below surface.			Similar reactions were observed for each of the other two organisms.
Nitrate reduction	±	±	+	
H_2S test	–	–	–	
Methyl red	±	Sl.+	+	
Voges-proskauer	–	–	±	

*a, Fermentation.
Sl.a. Slight acid without gas.

inguinale. The lung and gastrointestinal tracts grossly showed no lesions that could be considered the cause of death. Sections were not taken. Because this organism had resembled the Friedländer's bacillus in many respects, we performed comparative agglutination, absorption and precipitation tests between this organism and strains of Type II pneumococci. The results show that this organism is not similar to the Type A, B, or C of Friedländer's bacillus. However, we could not exclude it from the heterogeneous Group X of Friedländer's bacilli.

Guinea pigs and mice were also injected intraperitoneally with mixed cultures of these isolated organisms and gonococci. The results were similar to those obtained in the animals infected with the isolated organism alone.

DISCUSSION

We have observed in the nine cases which we recently studied here, and a review of many of the reported cases, that a concomitant gonorrheal and an occasional chancroidal infection were frequently present or were revealed in the history as existing prior to the beginning of the symptoms of granuloma inguinale. It is also noted that frequently an operation for inguinal adenitis (buboes) was performed, which did not heal.

The two cases reported by Gruhzit[9] both gave a history of inguinal swelling which failed to heal; one after lancing and the other after pricking. A similar history is given by Shattuck, Little and Coughlin[10] in a report of three cases from Boston. The extension of this unhealed incision is common in the history of granuloma inguinale cases.

The tissue transplantation en masse from one person with the disease to another susceptible person as done by McIntosh,[5] does not permit one to draw any conclusion as to the specificity of the organisms involved, since several organisms may be present.

A survey of the reported cases shows a higher incidence among colored than white of granuloma inguinale in the United States. This is a statement of fact, the explanation of which is involved in a large number of contributory factors. Of the contributory factors, the most important in this malady as in syphilis is the greater lack of information in the nature of personal hygiene among the colored.

SUMMARY AND CONCLUSION

We have isolated identical organisms from three cases of clinical granuloma inguinale. These organisms resemble organisms of the Friedländer's group and appear similar to the inclusion bodies in cells of curettements taken from ulcerations of clinical cases of granuloma inguinale.

We believe that the "Donovan bodies" are bacteria. The organism is nonpathogenic for mice, which is a differential point from Type A or B of the Friedländer's bacillus.

I wish to express my appreciation to Mr. Malaku E. Bayen and Miss Ferris Warren, juniors in the Medical and Dental Schools, for their assistance in most of these biochemical tests.

REFERENCES

1. Donovan, C.: Ulcerating Granuloma of the Pudenda, Indian M. Gaz. **40**: 414, 1905.
2. Flu, P. C.: Die Aetiologie des Granuloma Venereum, Beihefte z. Arch. f. Schiffs u. Tropen Hyg. **9**: 87, 1911.
3. Walker, E. L.: The Etiology of Granuloma Inguinale, J. M. Res. **37**: 427, 1918.
4. Poindexter, H. A.: Granuloma Inguinale, Am. J. Trop. Med. **14**: No. 2, 1934.
5. McIntosh, J. A.: Etiology of Granuloma Inguinale, J. A. M. A. **87**: 996, 1926.
6. Campbell, M. F.: Etiology of Granuloma Inguinale With Report of Eighteen Cases, Am. J. M. Sc. **174**: 670, 1927.
7. Randall, A., Small, J. C., and Belk, W. P.: Granuloma Inguinale, Surg. Gynec. Obst. **34**: 717, 1922.
8. DeMonbreun, W. A., and Goodpasture, E. W.: Etiology of Granuloma Inguinale, Am. J. Trop. Med. **13**: 447, 1933.
9. Gruhzit, O. M.: Granuloma Inguinale, Am. J. Trop. Med. **4**: 298, 1923.
10. Shattuck, G. C., Little, H. G., and Coughlin, W. F.: Treatment of Inguinal Granuloma, Am. J. Trop. Med. **6**: 307, 1926.

STEREOSCOPIC ROENTGENOGRAPHY OF BEDRIDDEN PNEUMONIA PATIENTS*

PAUL C. HODGES, PH.D., M.D., AND THOMAS H. LIPSCOMB, M.D., CHICAGO, ILL.

INVESTIGATORS in the field of pneumonia and physicians responsible for the care of patients suffering from this disease are generally aware of the great diagnostic value of roentgenograms. They need the help of the roentgenologist at the beginning of the disease, throughout its course and during convalescence, and in our experience are glad to accept him as an equal partner in the diagnostic problem. However, even the briefest experience in such work will convince any physician, whether he be roentgenologist or clinician, that the problem is difficult and the results not all that might be desired.

Early in the disease and again during convalescence the patient is able to come to the x-ray laboratory, sit up in front of a cassette changer and hold his breath during the making of standard quality stereoscopic films. This is impossible or at least highly undesirable during the active phase. Instead it has become common practice to bring a portable unit to the bedside or move the patient and bed to the x-ray laboratory and make single nonstereoscopic films with the patient recumbent. Such films are sometimes of astonishingly good quality but the lack of three-dimension vision is a serious handicap and the insertion of a cassette beneath the patient involves considerable discomfort for him.

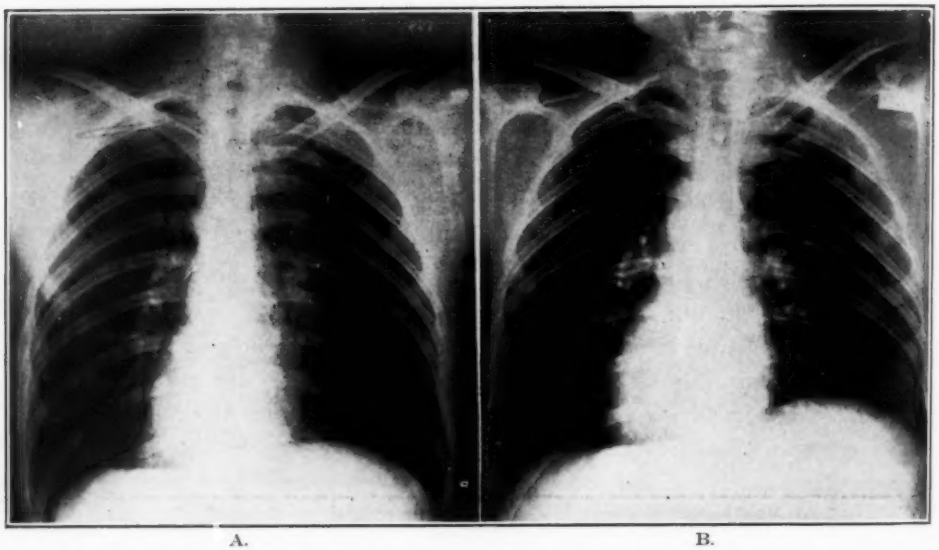
Clinicians not intimately associated with the problem are apt to underestimate the degree of this discomfort, holding the erroneous belief that an x-ray examination of the chest is an innocuous procedure if the patient does not leave his bed. The patient knows better. He must be lifted into a partial sit-

*From the Division of Roentgenology, Department of Medicine, University of Chicago.
Received for publication, May 22, 1934.

ting position while a cassette is inserted behind his back, and when he is lowered into the recumbent position he finds this an uncomfortable resting place even in that rare instance in which correct position is obtained at the first trial and rearrangement of patient and film is unnecessary.

In the course of many years of association with O. H. Robertson, whose investigations lie largely in the field of clinical and experimental pneumonia, one of us has made repeated attempts to improve the technical conditions of chest raying during the active stage of pneumonia.

Most of our earlier devices were found to involve too much effort on the part of the patient but the apparatus here described allows us to make stereoscopic chest films of recumbent subjects without submitting them to appreciable exertion or discomfort. In spite of the fact that the rays pass through mattress



A. B.

Fig. 1.—Roentgenograms of normal subject. H. H., aged thirty-three, x-ray No. 2297. A, (Serial 15) Regular technic, subject standing facing commercial cassette changer, 70 KV, 100 Ma., 72″ distance, exposure time 6/20 second.

B, (Serial 13) Subject on back in pneumonia bed, radiation from beneath through mattress, 70 KV, 150 Ma., 72″ distance, exposure time 8/20 second.

and bed clothing, films made under these conditions compare very favorably with those made in the conventional fashion, that is, with the subject standing facing a commercial cassette changer (Fig. 1).

As soon as a patient is suspected of having pneumonia he is transferred to a special pneumonia bed which he continues to occupy until there is no further need of special chest raying. This is a hospital bed* in which a square section of the upper half of the bed spring has been cut away and replaced by a square of stretched canvas (Fig. 2). An ordinary cotton-felt mattress rests on the

*These beds, originally in general use throughout our hospital, have recently been replaced by a newer type equipped with inner spring mattress and two crank-driven worm devices for raising and lowering head and foot. We have not yet succeeded in adapting these new beds for x-ray use because of the interfering shadows of coil springs and the cross-bars of the worm mechanism. We do not consider such adaptation impossible, particularly if it is undertaken by the manufacturer of the bed.

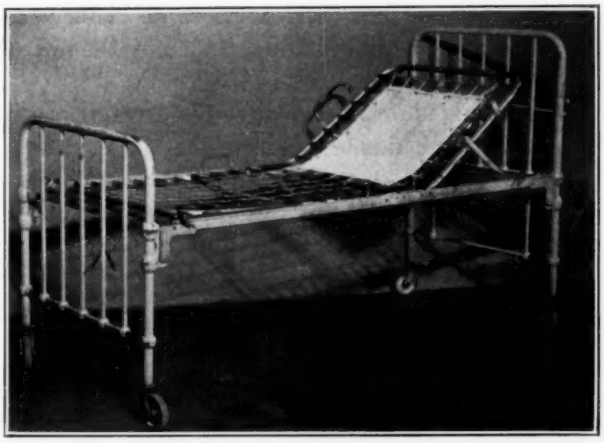


Fig. 2.—Pneumonia bed. A conventional hospital bed in which a section of spring has been cut away and the space filled in with a laced canvas window. In more modern type beds with worm mechanism for raising and lowering the head, rearrangement of the mechanism is necessary in order to provide a clear space beneath the chest. A regular cotton-felt mattress is used, care being taken that it contains no bits of wire or other metal objects.

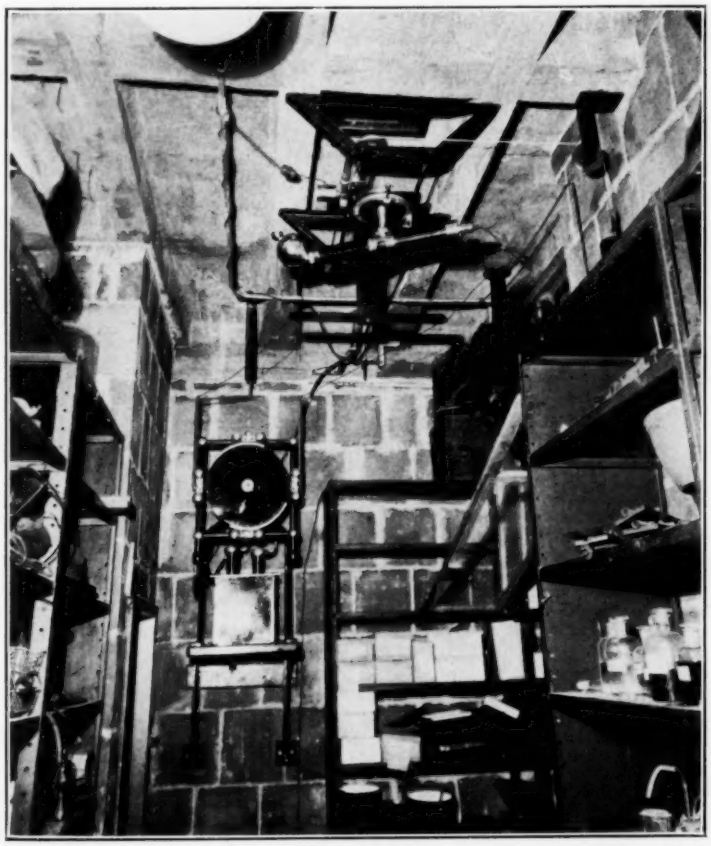


Fig. 3.—X-ray tube suspended from ceiling in lower room. The cocking device for the stereoscopic shift, machine controls, etc., are in the room above. Protective wire caging has been removed to allow photographing the apparatus.

spring and except for the fact that there must be no rubber sheet beneath the patient's chest, the bedding is arranged and changed by the nurses in accordance with ordinary hospital practice.

Two x-ray rooms are required, one directly above the other. The lower room contains a mechanical rectifier x-ray machine mounted on a wall shelf together with a water-cooled 10 KW x-ray tube slung from a special stereoscopic tubestand attached to the ceiling (Fig. 3). The upper room, capable of darkening for fluoroscopy, contains the control stand and meters of the x-ray machine

Fig. 4.—Patient on pneumonia bed in position for stereoscopic filming. The fluoroscopic screen is intended primarily for centering but with the addition of a shutter may be used for fluoroscopic observation of diaphragmatic excursion, etc.

and a special automatic cassette changer counterbalanced for easy vertical movement on two vertical rails. A rectangular, bakelite-covered opening through the floor transmits radiation from the tube lying directly beneath it, the tube target, center of floor port and center of film lying on a straight line perpendicular to the floor (Fig. 4). The patient recumbent on the pneumonia bed is wheeled into position above the floor port in the upper room, his head is turned to one side and the lowered cassette changer is brought down until the bakelite front of its central section touches the chest. The room is now darkened and

under fluoroscopic control, using radiation coming through the floor and a screen mounted on the upper surface of the central section of the cassette changer, the bed is moved about until the patient's chest is properly centered beneath the space presently to be occupied in succession by the two films.

During this fluoroscopic centering, which takes but a few seconds, the films are protected by the lead floor of the two outer sections of the changer. One cassette carriage is cocked against spring pressure and held by a stout trigger. The other, except for the light pressure of a friction catch, is free to roll along its track. This free carriage is now shoved into position between the bakelite window and fluorescent screen of the central section where it engages the cocked carriage and becomes attached to it, the machine controls are turned to the proper setting for filming and by means of a cable-and-lever arrangement the tube is cocked against spring pressure. It is now possible to make stereoscopic chest films employing speeds of exposure and film change about the same as those used with conventional vertical type cassette changers.

There are magnetic releases on tubestand and cassette changer and a motor-driven time switch times the exposures. Operation is further simplified by a five-pole, double-throw, externally operated switch. With the switch handle in the down position, the time-switch motor is off and the tube is set for long-distance fluoroscopy at 85 KV and 10 Ma. When the switch handle is up, the time-switch motor runs and the tube is ready to deliver 150 Ma. at 70 KV.

The apparatus meets a definite though limited need in our service. It seems to satisfy completely the requirements of stereoscopic chest filming at any phase of pneumonia unless pleural fluid be present and, in addition, renders valuable assistance in postoperative conditions where the clinical picture is complicated and it is important to know whether pneumonia or pulmonary infarct is present. To those who employ artificial pneumothorax in the treatment of pneumonia the apparatus offers a means for the frequent checking of the amount of pleural air and the degree of pulmonary collapse.

One of the leading x-ray manufacturers has constructed a neat and compact commercial model of our apparatus* and commercial manufacturers of hospital beds could undoubtedly develop beds that would meet the x-ray requirements and at the same time be more comfortable than our homemade product.

*For the University of Michigan.

THE RESPONSE TO INFECTION IN BONE MARROW DYSCRASIAS*

FRANK H. BETHELL, M.D., ANN ARBOR, MICH.

CERTAIN changes in the constitutional environment of an individual, notably those caused by bacterial invasion, produce a more or less characteristic response on the part of the bone marrow. The neutrophile count provides a measure of the degree of such response. The functional capacity of the bone marrow to meet a sustained demand for more than usual numbers of neutrophiles is evidenced not only by the total number of neutrophiles per cubic millimeter but also by their stage of maturity. It is this conception which forms the basis of the Schilling[1] index and its interpretation. More recently the importance of immature white blood cell counts in infectious conditions has been emphasized by Reznikoff.[2] He points out that in many serious infections there is no increase in the circulating neutrophiles, but rather an apparent depression of the myelocytic response. The decrease in leucopoietic activity may be the specific effect of certain diseases, such as typhoid fever, tuberculosis, influenza, and malaria, or it may follow prolonged overstimulation in response to an overwhelming infection such as streptococcal septicemia. During recent years great interest has been shown in the phenomenon of extreme neutropenia which may develop in the presence of little or no demonstrable infection. If, as often happens in such cases, severe infection supervenes, the few neutrophiles found are predominantly immature in form. It is the relative percentage of immature and adult forms which indicates the effort of the bone marrow response as opposed to the total neutrophile count which measures solely the extent of the response.

The appearance in the blood stream of increasing numbers of young neutrophiles during the progress of an infection is dependent upon two factors, the state of the individual prior to the present illness, as reflected in the bone marrow, and the nature of the infection. Consequently, there are actually two variables, but in order to interpret the progress of an infection by studies of the neutrophile age the bone marrow must be thought of as a constant. In many instances, however, the neutrophile picture differs widely from what one would expect from the clinical course of the illness. The cases reported by Thompson,[3] in which there was either an overproduction of young and abnormal white blood cells or a marked decrease in the numbers of granulocytes in response to infection, fall into this group. The patients to be discussed in the present communication, likewise, showed an atypical bone marrow response in the presence of infection.

*From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan.

Received for publication, May 11, 1934.

A summary of this paper was read at the New York meeting of the American Society of Experimental Pathology, on March 31, 1934.

While the diagnostic and prognostic value of the Schilling index and its modifications is limited to such cases as show a characteristic bone marrow response, the occurrence of cytoplasmic changes in the neutrophiles, and especially the appearance of granules which are basophilic to one of the Romanowski stains, is quite independent of the type of myelocytic reaction and will, in any case, give a reasonably accurate picture of the progress of an acute severe infection. Alterations in the staining characteristics of the cytoplasms of leucocytes have received considerable attention among German ingestigators,[4, 5, 6, 7] but elsewhere they have met with little systematic study except by Rosenthal and his coworkers.[8, 9, 10, 11] The reader is referred to these authors for morphologic descriptions of the phenomena under consideration. Basophilic granulation of the neutrophiles is not limited to infectious processes, but may occur in a wide variety of conditions in which there is marked alteration of the individual's internal environment with evidence of toxemia. Among such conditions may be mentioned reactions following transfusion with incompatible blood, intensive roentgen therapy, the toxemias of pregnancy, extensive malignancy with necrosis, and chronic myelogenous leucemia.

That these granules, in infectious states, are not merely a degenerative phenomenon, caused by exposure of the cells to a site of inflammation, is attested by the evidence gained from counts made on successive days during the exacerbation of an infection. When the total number of immature and mature forms exhibiting basophilic granulation are compared, it is shown that the granules are predominant in the cells most recently released into the circulation, and consequently, in all probability, the change occurs in the bone marrow. Table I illustrates a method by which the neutrophile counts may be tabulated

TABLE I

NEUTROPHILE COUNTS MADE ON THREE SUCCESSIVE DAYS SHOWING INCREASE IN PERCENTAGE AND ABSOLUTE NUMBER OF IMMATURE FORMS EXHIBITING CYTOPLASMIC BASOPHILIC ("TOXIC") GRANULATION. THE COUNTS WERE MADE ON CASE 1

DATE	MARCH 9		MARCH 10		MARCH 11	
	PER CENT	NO. PER C.MM.	PER CENT	NO. PER C.MM.	PER CENT	NO. PER C.MM.
W.B.C. per c.mm.		4,800		7,100		9,450
Percentage of nonfilament neutrophiles	45		51		57	
No. per c.mm. nonfilament neutrophiles		2,160		3,620		5,390
Percentage of filament neutrophiles	45		41		41	
No. per c.mm. filament neutrophiles		2,160		2,910		3,870
Total percentage of neutrophiles	90		92		98	
Total no. per c.mm. of neutrophiles		4,320		6,530		9,260
Percentage of nonfilament forms with basophilic granulation	26*		39*		58*	
No. per c.mm. of nonfilament forms with basophilic granulation		1,120		2,530		5,400
Percentage of filament forms with basophilic granulation	28*		27*		14*	
No. per c.mm. filament forms with basophilic granulation		1,210		1,750		1,320
Total percentage of neutrophiles with basophilic granulation	54*		66*		72*	
Total no. per c.mm. neutrophiles with basophilic granulation		2,330		4,280		6,720

*These percentages are of the total number of neutrophiles per cubic millimeter on the respective days.

in order to correlate the nuclear shift with the percentage and absolute number of basophilic granule cells. These counts were made on the patient reported as Case 1.

Blood examinations on the patients comprising the present study were made at frequent intervals throughout the period of hospitalization, and during the acute manifestations of their illness counts were taken daily. For purposes of simplification only the more significant findings will be reported. Leucocyte counts were done with pipettes and hemocytometers certified by the U. S. Bureau of Standards. Films were made on cover slips and stained with a fresh solution of Wright's stain, prepared in the usual manner without buffering. Control stains on blood from individuals without acute infection, or other evidence of toxemia, were carried out routinely. The polymorphonuclear neutrophiles were classified according to their nuclear structure into nonfilament and filament groups as suggested by Cooke and Ponder.[12] However, the percentage recorded is of the total number of neutrophiles rather than of the total leucocyte count. On this basis 6 per cent of nonfilament forms is an average normal value. On the charts only the nonfilament percentage is plotted, and this value, "the percentage of immaturity," is regarded as a measure of the effort of the bone marrow response. For graphic purposes basophilic granulation is recorded in terms of the percentage of neutrophiles exhibiting this phenomenon, rather than in the form of a "degenerative index" as advocated by Kugel and Rosenthal.[9] The tabulation of at least 100 neutrophiles was included in each differential. In the severe neutropenic states this usually required the examination of several films.

By the use in conjunction of the three lines of inquiry enumerated above, namely, the degree of bone marrow response as measured by the total neutrophile count, the effort of the response as indicated by the percentage of immature forms, and the severity of the infection as interpreted by the percentage of basophilic granule cells, it is hoped that some information may be gained concerning the physiology of the bone marrow in certain pathologic states. Incidentally, these studies confirm the prognostic value of the basophilic granule cell count.

CASE REPORTS

CASE 1.—M. N. Acute pansinusitis, cavernous sinus thrombosis and terminal streptococcal septicemia, without evidence of primary disease of the blood-forming organs. An American housewife of forty-eight years entered the hospital complaining of difficulty of breathing, cough, and weakness. For four years she had been subject to attacks of otitis media and acute sinusitis. Right mastoidectomy was done. The present illness commenced four months before admission with sore throat and right-sided frontal headache. Upper respiratory symptoms became increasingly severe, and during the two weeks prior to her entering the hospital small nodules developed in the skin of the trunk and extremities. On examination the patient appeared to be seriously ill. There was a mucopurulent discharge in the nose and throat and thickening of the mucosa of the nares and pharynx. The right membrana tympani was dull and reddened. Scattered over the trunk and extremities were numerous small cutaneous lesions with depressed bluish centers, surrounded by elevated yellow areas, which, in turn, were circled by zones of erythema. Temperature was 98° F.; pulse, 100; respirations, 27. The patient's condition did not improve, the temperature was continuously "septic" in type, localized suppuration occurred in the right periorbital tissues and

in the region of the right elbow. On the twentieth day of hospitalization she died. The red blood cell count on admission was 3,700,000 per c.mm.; hemoglobin, 59 per cent (Sahli); white blood cells, 7,300 per c.mm. Polymorphonuclear neutrophiles were 78.5 per cent; eosinophiles, 2.5 per cent; large lymphocytes, 15.5 per cent; small lymphocytes, 2.5 per cent; monocytes, 1 per cent. On the day of death the white blood cell count fell to 1,750 with a neutrophilic percentage of 94. Leucocyte and neutrophile counts made on three successive days on this patient are given in Table I, and the character of the neutrophile response is plotted on Chart 1. The significant findings at necropsy in this case were streptococcus septicemia with focal necroses in skin, lungs, lymph nodes, spleen and liver, and acute parenchymatous degeneration of all organs. Microscopic examination of the marrow of the sternum and ribs revealed a congested mixed cellular and fatty marrow with numerous giant cells, and large areas nearly devoid of cells. The marrow of the ethmoid was almost completely exhausted of cellular elements with areas of necrosis and hemorrhage.

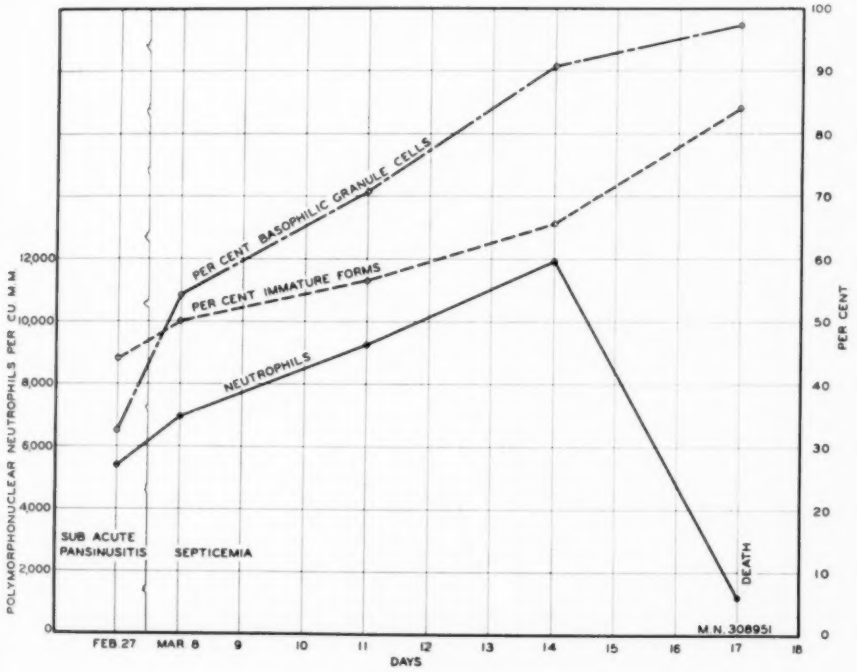


Chart 1.

This case is presented as an example of failing bone marrow response to an overwhelming infection. As no primary deterrent to leucopoiesis exists the effort of the marrow response, as measured by the percentage of nonfilament neutrophiles, parallels the severity of the infection, plotted in terms of the percentage of neutrophiles exhibiting basophilic granulation. The observations on this patient are to be contrasted with those made on cases whose infection complicated a primary bone marrow dyscrasia.

Case 2.—S. M. Pernicious anemia in relapse, acute otitis media with mastoiditis, and acute cholecystitis and cholelithiasis. An American woman of fifty-two years, self-supporting, was admitted with a complaint of weakness, numbness and tingling of the extremities, and recurrent soreness of the tongue. The present illness was of eleven months' duration, the symptoms becoming gradually more severe. Four months before entering the hospital she began to suffer with attacks of pain in the right upper abdominal quadrant, accompanied by

nausea and vomiting, and at times followed by transient jaundice. Examination revealed pallor and icteric tint of the skin and mucous membranes, tenderness in the right hypochondrium, enlargement of the liver, and impairment of vibratory sense in the lower extremities. Temperature 100° F.; pulse 80; respirations 22. During the first three weeks of hospitalization tenderness and pain in the right hypochondrium persisted, there was an irregular fever of moderate degree, and no significant response on the part of the blood following treatment with ventriculin and intramuscular liver extract. Subsequently the patient complained of right earache; the drum was found to be reddened and bulging, and paracentesis was done with the release of much mucopurulent exudate. Tenderness over the right mastoid developed, and the temperature became markedly "septic" in character. The patient's condition was considered to be too poor for operation; blood transfusions were given with slight temporary improvement, but all efforts to overcome the infection were unavailing, and five weeks after admission she died. A necropsy was not obtained.

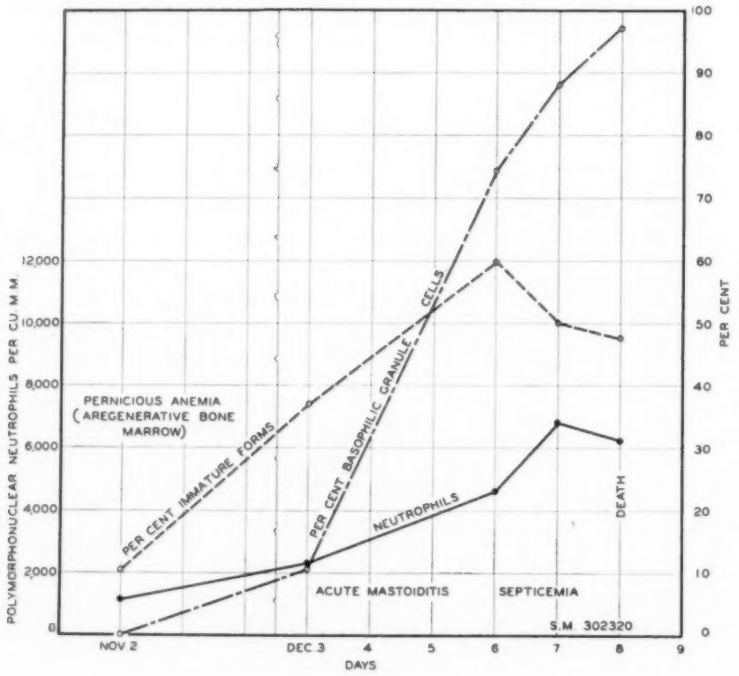


Chart 2.

The red blood cell count on admission was 1,130,000 per c.mm.; hemoglobin, 29 per cent (Sahli); white blood cell count, 3,000 per c.mm.; polymorphonuclear neutrophiles were 38.5 per cent; large lymphocytes, 17 per cent; small lymphocytes, 43.5 per cent; monocytes, 1 per cent. The stained blood film revealed the morphologic characteristics of pernicious anemia with definite macrocytosis of the erythrocytes. Achlorhydria of the gastric contents was found after the subcutaneous injection of histamine hydrochloride 1.0 mg. Cholecystography following the intravenous administration of dye revealed nonvisualization of the gallbladder with multiple opaque stones. During the course of the infection there was a very limited increase in white blood cells, the maximum count being 8,350 with 83 per cent neutrophiles two days before death. On Chart 2 are plotted the total neutrophile counts, percentages of nonfilament forms, and percentages of basophilic granule cells. This chart depicts an inadequate response dependent not upon the degree or duration of the infection but upon functional derangement of the bone marrow.

In the relapse phase of pernicious anemia there is decreased productivity of all myeloid elements, involving the leucopoietic and platelet forming as well as the erythropoietic tissues. Sturgis, Isaacs, and Smith[13] and Smithburn and Zerfas,[14] among others, have shown that the presence of infection may inhibit the expected response to therapy in this disease. The bone marrow, consequently, may remain functionally incapable of reacting adequately to the infective process. Neither the degree nor the effort of the response is commensurate with the severity of the infection. In such a case the ordinary hemogram would be of little prognostic value.

CASE 3.—E. B. Pernicious anemia in beginning remission, acute infection (? encephalitis). An American born male of English-German descent, aged sixty-two years, and by occupation a photographer, entered the hospital complaining of weakness and persistent

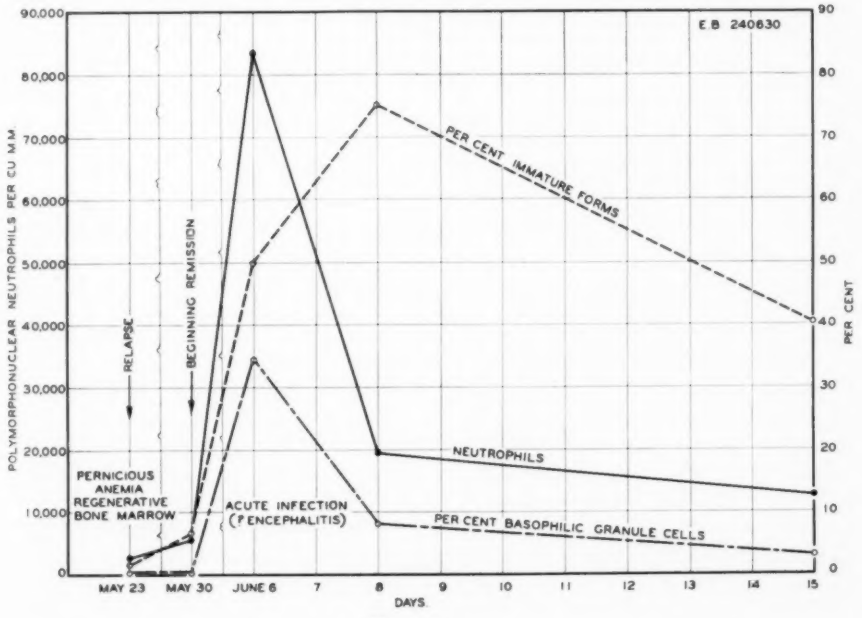


Chart 3.

numbness and tingling of the fingers and toes. His symptoms were of gradual onset and progression and had been first noticed about one year prior to his admission. Examination disclosed pallor and slight icterus, atrophy of the papillae of the skin, palpable liver and spleen, and impairment of vibratory sense and sense of motion and position in the lower extremities. Temperature was 99.8° F.; pulse, 88; respirations, 20. Ventriculin therapy was instituted and there occurred a typical hematologic response, accompanied by symptomatic improvement. However, thirteen days after admission the patient became acutely ill with moderate fever and marked leucocytosis. There was no localization of symptoms, extreme drowsiness and mental confusion being the only subjective manifestations. No objective signs were present; urinalysis, blood culture, and agglutination tests were negative; the lung fields were clear by roentgen examination; and lumbar puncture revealed only slightly increased pressure with normal spinal fluid. Acute encephalitis and subdiaphragmatic abscess were suggested respectively by neurologic and surgical consultants, and of the two conditions, the former, in the light of the subsequent course, appears more plausible. Within two weeks of its onset all evidences of the infection had disappeared, and remission of the anemia continued satisfactorily.

On admission the red blood cell count was 1,440,000 per c.mm.; hemoglobin, 36 per cent (Sahli); white blood cells, 4,350 per c.mm. Polymorphonuclear neutrophiles were 60 per cent; eosinophiles, 2 per cent; large lymphocytes, 11.5 per cent; small lymphocytes, 24.5 per cent; monocytes, 2 per cent. Achlorhydria of the gastric contents was present. Following ventriculin therapy the reticulocytes attained a maximum of 27 per cent. Subsequently, with the development of infection, the leucocyte count rose rapidly to 94,200 per c.mm. with a neutrophile percentage of 88. Significant neutrophile counts, percentages of immature forms, and percentages of basophilic granule cells for this case are given on Chart 3. It will be seen that the degree and effort of the bone marrow response are out of all proportion to the evidences of infection.

Fleming[15] and Heck and Watkins[16] have shown that the neutropenia and high percentage of segmented forms (shift to the right), which are commonly found in pernicious anemia in relapse, are replaced by a normal neutrophilic picture during therapeutically induced remission. In uncomplicated cases of pernicious anemia the increase in neutrophiles and alteration of the nuclear shift, occurring in remission, are rarely of marked degree. However, if an infection develops during or soon after the period of active reticulocytosis there occurs an extraordinary neutrophile response. Large numbers of nonfilament forms are discharged into the circulation and the immature cells may continue to predominate for some time after the infection has begun to subside. It appears that, in such cases, the bone marrow is in a hyperactive and supersensitive state, so that the stimulus of even a moderate infection elicits a maximal response.

Case 4.—E. F. Postarsphenamine aplastic anemia and granulocytopenia; gangrenous stomatitis; latent syphilis. An American man, of forty-eight years, a construction engineer, was admitted complaining of weakness and bleeding from the gums. He was known to have had syphilis for twelve years and had received numerous courses of arsphenamine and neoarsphenamine therapy, together with other antisyphilitics. No clinical manifestations of his disease were present, but the Wassermann test persistently remained positive. Beginning six months before his admission he received 18 weekly injections of neoarsphenamine. After the fifteenth a rash developed over the legs; after the eighteenth, one month prior to admission, he experienced increasing weakness and shortness of breath. The treatment was discontinued, but he became progressively weaker, and noticed recurrent oozing of blood from the gums. He entered the hospital acutely ill; there was marked pallor of the skin; an exfoliative maculopapular eruption was present over the lower extremities and back; subretinal hemorrhages were seen; the pharynx was reddened with a considerable amount of mucoid exudate.

While in the hospital a tender swelling developed in the right lower alveolus. The lesion rapidly extended, and the temperature became markedly "septic" in type. There was little inflammatory reaction about the infective process in the mouth. Ultimately massive necrosis of the oral tissues developed, and on the twenty-sixth day of hospitalization the patient died.

The red blood cell count on admission was 1,300,000 per c.mm.; hemoglobin, 18 per cent (Sahli); white blood cells, 3,250 per c.mm. Polymorphonuclear neutrophiles were 22 per cent; eosinophiles, 1 per cent; large lymphocytes, 33 per cent; small lymphocytes, 40 per cent; monocytes, 4 per cent. The platelets were greatly reduced in number. During the period of acute infection the leucocyte count steadily declined, and on the day preceding death the count was 750 per c.mm. with 24 per cent neutrophiles. The findings are recorded on Chart 4. The necropsy revealed syphilitic myocarditis, aortitis, and adrenalitis, gangrenous stomatitis and glossitis, multiple small internal hemorrhages, acute fibrinous pericarditis, and terminal lobular pneumonia. There was an almost complete fatty replacement of the cellular marrow of the sternum, ribs, and vertebrae.

A depressing effect upon the bone marrow of the arsenicals used in treating syphilis has long been recognized and the subject is well discussed by Farley.[17] Recently Loveman[18] has reviewed a number of reported cases of aplastic anemia following the administration of arsphenamine and related compounds. It is of interest that in the case presented here the percentage of basophilic granule cells parallels the clinical course of the infection, although no evidence of bone marrow response is obtained either from the total neutrophile counts or the determinations of the percentages of immature forms.

CASE 5.—E. E. Agranulocytic angina. A graduate nurse of twenty-six years was admitted to the otolaryngologic service complaining of severe sore throat of two days' duration.

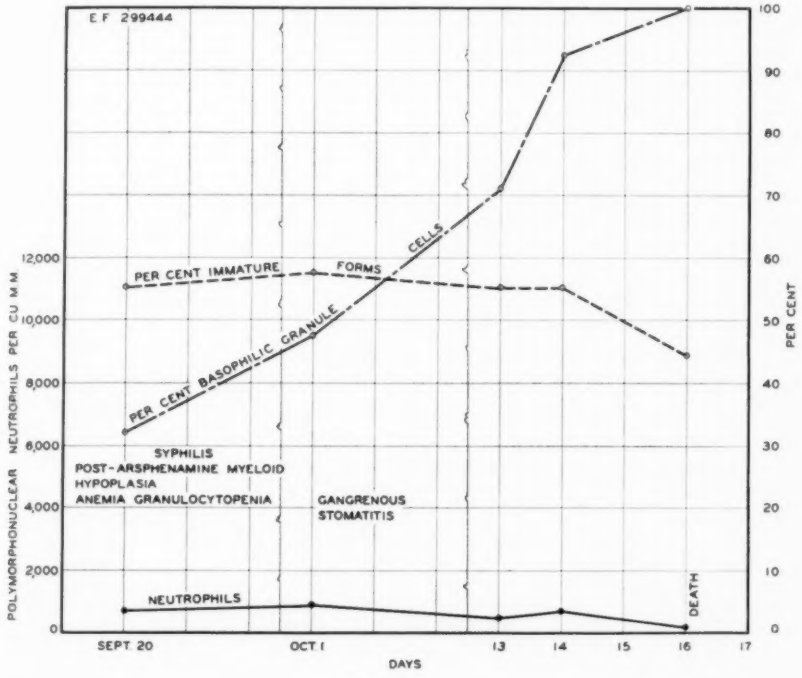


Chart 4.

Over a long period of time she had been accustomed to taking amidopyrine and barbital for the relief of dysmenorrhea and chronic sinusitis. For five days preceding the onset of the sore throat she had taken 0.3 or 0.6 gm. of amidopyrine three times daily. On examination she appeared to be acutely ill. The pharynx and tonsils were intensely reddened with patches of gray exudate. Temperature was 99.2° F.; pulse, 108; respirations, 24. The temperature rapidly rose to over 103° and became "septic" in character. Leucopenia with marked reduction in the neutrophiles was discovered and on the day following admission she was transferred to the medical service. Over a period of five days she was given 10 intramuscular injections each of 10 c.c. of 7 per cent pentnucleotide solution. During this time the infectious process in the throat progressed and areas of necrosis developed. After the fifth day of therapy there occurred a neutrophilic response and the local condition in the throat improved. However, signs of sepsis persisted and as the neutrophile count failed to increase appreciably after its initial rise administration of pentnucleotide was resumed. Ten days after admission the patient developed localized bronchopneumonia and pleurisy with effusion.

This was followed by empyema. She was transferred to the Department of Thoracic Surgery where open drainage was done. After a stormy convalescence she was able to leave the hospital fifty-four days after her admission.

On the day of transfer to the medical service the red blood cell count was 4,300,000 per c.mm.; hemoglobin, 70 per cent (Sahli); white blood cells, 800 per c.mm.; polymorphonuclear neutrophiles, 8 per cent. Two days later the leucocyte count was 450 per c.mm., polymorphonuclear neutrophiles, 27 per cent; large lymphocytes, 38 per cent; small lymphocytes, 31 per cent; monocytes, 4 per cent. On the fifth day of pentnucleotide therapy the white blood cell count was 3,050 of which 32 per cent were neutrophiles. Subsequently, at the height of the pneumonic process, the count increased to a maximum of 22,000, 83 per cent neutrophiles. On Chart 5 are plotted a few of the significant neutrophile observations in this case. The intervening counts are, in general, in accord with the curves shown. It is not intended to give a detailed record of this patient's neutrophilic reaction during her illness, but rather

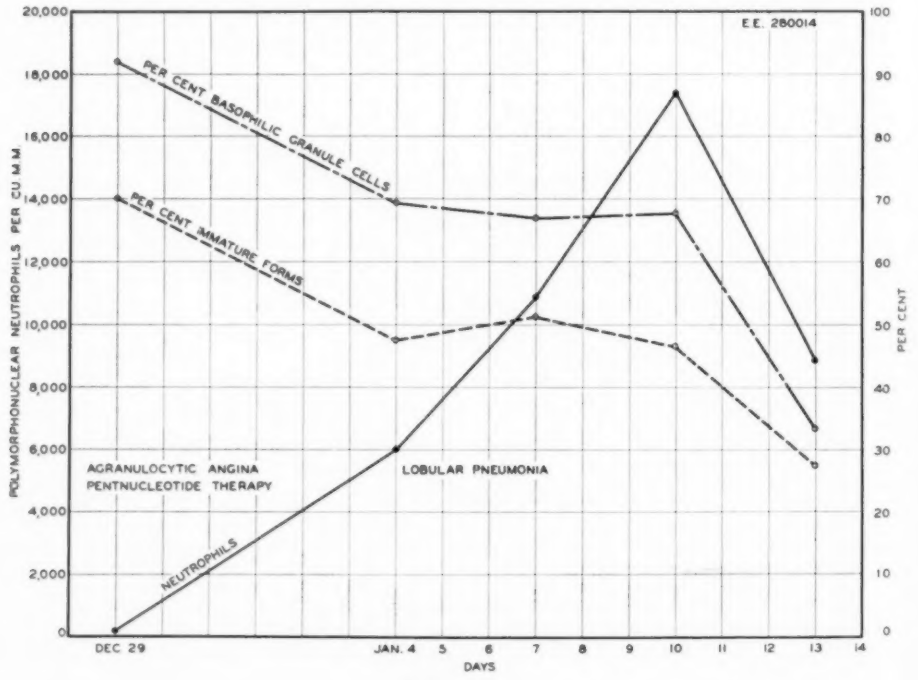


Chart 5.

to portray the character of the reaction. It should be observed that during the first period of the illness, coincident with the acute pharyngitis, there is a high percentage of basophilic granule cells without neutrophilia. Later, with the onset of pneumonia, the percentage of basophilic granulation again rises, but the factor interfering with response having been removed, there is a simultaneous increase in the total number of neutrophiles.

It has long been known that chemicals possessing the benzene ring may depress bone marrow function, and Kracke,[19] from experimental evidence, had reason to believe that benzene compounds, in certain circumstances, exerted their action specifically on the leucopoietic elements of the bone marrow. Selling,[20] much earlier, was able to produce a leucopenia, without anemia, in rabbits by injecting benzene subcutaneously. However, it was not until the past year, following the reports of Madison and Squier[21] and of Watkins,[22] that a definite connection was established between severe neutropenia and the admin-

istration of certain drugs commonly employed in the practice of medicine. Of these the most conspicuous offender is amidopyrine. The action of this drug can only be surmised at present. The commonly accepted view is that in some individuals an idiosynerasy to amidopyrine and related compounds exists with a resulting allergic reaction affecting the leucopoietic elements of the bone marrow. However, the high percentage of immature forms, in spite of the neutropenia, indicates that the bone marrow is at least capable of making an effort to respond. The explanation may lie, as suggested by Isaacs,[23] in the induction by the drug of an increased excretion of the neutrophiles into the alimentary tract.

Case 6.—F. P. Typhoid fever. Extreme neutropenia. A young woman of twenty years, born in America of Polish ancestry, was transferred from the psychopathic service of the University Hospital to the Simpson Memorial Institute complaining of extreme malaise, feverishness, and lower abdominal pain. Six weeks previously she had been admitted to the psychopathic institution with a "psychiatric status typical of crude conversion hysteria in a feeble-minded individual." The medical history was not significant except that two months before her admission she contracted a severe upper respiratory infection with sore throat and fever. This illness lasted two weeks and left her weakened, depressed, and emotionally unstable. The nature of the treatment given at this time is not known, and no blood counts were made. The patient's course while on the psychopathic service was uneventful. Two weeks after her admission twelve carious teeth were extracted without sequelae. No drug therapy was given. On the day preceding her transfer she complained of generalized lower abdominal pain, was nauseated and vomited. The temperature was 103.6° F. Repeated blood counts revealed a progressive reduction of white cells affecting particularly the neutrophiles, and on the evening following the onset of symptoms she was transferred. It is probable that the acute illness was of longer duration than the records indicate, but because of the patient's unresponsiveness it was not recognized earlier. The temperatures of patients on the psychopathic service are not taken routinely. On examination the patient appeared acutely ill; facies apprehensive and dull; pharynx definitely reddened, but without exudate; lungs clear; heart negative; generalized moderate tenderness over the lower abdomen with increased muscle tonus, but no localized rigidity. Temperature was 106° F.; pulse, 88; respirations, 32. In spite of no known source of infection (no instances of typhoid fever had occurred in the psychopathic institution for a number of years), typhoid fever was suspected and its presence confirmed by blood culture, and serum agglutination tests. In addition to the usual measures of treatment 10 c.c. of 7 per cent pentnucleotide solution was given intramuscularly twice daily for five days, because of the persistent extreme neutropenia. Later intramuscular liver extract was used. Two transfusions each of 500 c.c. citrated whole blood were given. No symptomatic improvement nor significant increase in the white blood cell count followed these therapeutic measures. Eight days after recognition of the acute illness she died, following evidences of intestinal hemorrhage.

At the time of transfer to the Simpson Memorial Institute the red blood cell count was 2,530,000 per c.mm.; hemoglobin, 50 per cent (Sahli); white blood cells, 650 per c.mm.; polymorphonuclear neutrophiles, 20 per cent; eosinophiles, 0; basophiles, 0; large lymphocytes, 31 per cent; small lymphocytes, 47 per cent; monocytes, 2 per cent. A tabulation of the leucocyte counts, including those made before the onset of the acute illness, is given in Table II. The character of the neutrophile reaction, both before and during the acute illness, is portrayed in Chart 6. Throughout the rapidly fatal course of the disease, and in the face of an increasing degree of basophilic granulation, there is little change in the character of the bone marrow response.

The necropsy findings in this case include typhoid fever in stage corresponding to second or third week; large celled hyperplasia of intestinal lymphoid tissue with ulcers in lower ileum, cecum, and rectum; localized peritonitis; hyperplasia of retroperitoneal lymph nodes

and spleen; focal necroses in liver; acute purulent bronchitis and bronchopneumonia. Microscopic examination of the marrow of the sternum, ribs, and vertebrae revealed a cellular marrow with a preponderance of erythroblastic elements.

This case of typhoid fever is remarkable because of the extreme neutropenia (130 neutrophiles per c.mm.); its rapid development; and its occurrence during the first week of the disease. Thayer[24] found the lowest of 832 leucocyte counts made on uncomplicated cases of typhoid fever to be 1,000 cells per c.mm., the lowest neutrophilic percentage, 24.6, but as the leucocyte and differential counts are not correlated it is difficult to use his data for comparative purposes. Austin and Leopold[25] reported a case of more severe neutropenia than the one presented here, but in their patient the neutropenia developed in the third week of the disease and was accompanied by a great increase of lymphocytes, attaining 98 per cent, or 8,448 per c.mm.

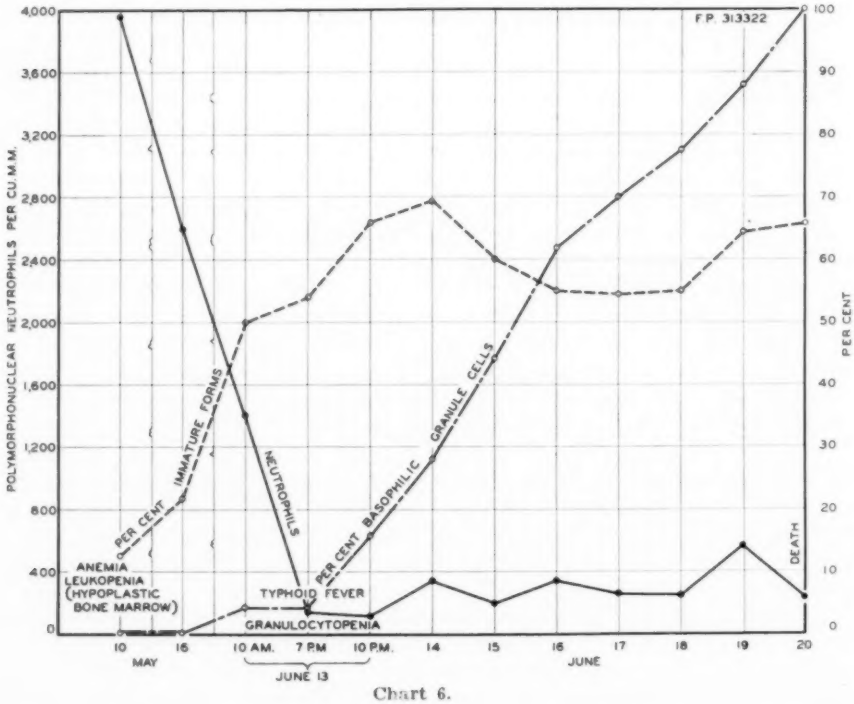


Chart 6.

The explanation of our patient's neutropenia probably lies in Schilling's[26] theory of leucopoietic paralysis by the toxemia of the infection with consequent "degenerative left nuclear shift." Whether the extreme effect was due to an especially virulent strain of *B. typhosus*, or to a primary functional defect of the bone marrow, it is impossible to say, but it is perhaps suggestive that the blood counts made before the acute illness revealed a reduction of all elements of bone marrow origin. There was a moderate degree of orthochromic anemia, a decrease in platelets, and a neutrophile count at or below the lower limit of normal. It may be justifiable to regard this case as one of constitutional hypoplasia of the bone marrow with unusual susceptibility to the toxic properties of the typhoid bacillus.

TABLE II

LEUCOCYTE COUNTS ON CASE 6

DATE	W.B.C. PER C.MM.	PER CENT							
		META-MYELO-CYTES	NONFILA-MENT NEUTRO-PHILES	FILA-MENT NEUTRO-PHILES	EOSINO-PHILES	BASO-PHILES	LARGE LYMPHO-CYTES	SMALL LYMPHO-CYTES	MONO-CYTES
5/10/33	6,100	0	8.0	56.0	1.0	1.0	18.0	11.0	5.0
5/16	4,900	0	12.0	41.0	2.0	0	25.0	18.0	2.0
6/13 11 A.M.	5,100	0.5	13.5	14.0	0	2.0	38.0	23.0	9.0
6/13 7 P.M.	600	1.0	12.5	11.5	0	0	36.0	23.0	16.0
6/13 10 P.M.	650	2.0	11.0	7.0	0	0	41.0	26.0	13.0
6/14	650	4.5	33.0	16.5	0	0	25.0	16.0	5.0
6/15	1,000	1.5	33.0	16.5	0	0	28.0	18.0	3.0
6/16	950	2.5	18.0	16.5	0	0	45.0	17.0	1.0
6/17	700	1.0	20.0	18.0	0	0	47.0	11.0	2.0
6/18	500	5.0	24.0	23.0	0	0	35.0	12.0	1.0
6/19	1,350	5.0	22.0	15.0	0	0	44.0	14.0	0
6/20	650	6.0	17.0	12.0	0	0	46.0	18.0	1.0

No attempt has been made to formulate theories concerning the mechanism of the bone marrow response to infection, based on the clinical material presented here. The foregoing cases are reported as unusual types of myelocytic reaction to infection, and are illustrative of the ways in which primary disease of the bone marrow, or of the internal factors regulating hematopoiesis, may alter the nature of the response.

CONCLUSIONS

The response of the bone marrow to infection is dependent upon its pre-existing functional state, as well as upon the nature and severity of the infectious process.

The estimation of the number of neutrophiles in the circulating blood and their classification into nonfilament and filament nuclear forms does not differentiate between the intrinsic and extrinsic factors involved in the response. The occurrence of basophilic granules in the cytoplasm of the neutrophiles is, on the other hand, solely a result of the infection.

The total neutrophile count may be regarded as a measure of the degree of the bone marrow response, the percentage of nonfilament or immature forms as an indication of the effort of the response, and the percentage of neutrophiles exhibiting basophilic granulation as a sign of the severity of the infection. By correlation of these data, obtained from successive blood examinations of the same individual, it is possible to ascertain the nature of the bone marrow response as well as the progress of the infection.

Six cases are reported of infection in the presence of various types of bone marrow dyscrasia which illustrate the manner in which this correlation is carried out. In these cases the percentage of neutrophiles exhibiting cytoplasmic basophilic granulation gives a more accurate indication of the severity and the progress of the infection than either the total number of neutrophiles per cubic millimeter or their degree of immaturity. Of the four fatal cases presented,

pathologic diagnoses were available on three, and so far as the methods of examination permitted, the conclusions arrived at by the blood studies were confirmed.

REFERENCES

1. Schilling, V.: Über die Notwendigkeit gründsätzlicher Beachtung der neutrophilen Kernverschiebung in Leukozytenbilde und über praktische Erfolge dieser Methode, Ztschr. f. klin. Med. **89**: 1, 1920.
2. Reznikoff, P.: Immature White Blood Cell Counts in Infectious Diseases, J. A. M. A. **93**: 963, 1929.
3. Thompson, W. P.: Abnormalities in the White Blood Cell Response (Leukemoid, Atypical Leukemic, and Leukopenic Blood Picture), Am. J. M. Sc. **3**: 334, 1931.
4. Naegeli, O.: Blut-Krankheiten und Blutdiagnostic, ed. 4, Berlin, 1923, Julius Springer.
5. Gloor, W.: Die klinische Bedeutung der qualitativen Veränderung der Leukozyten, Leipsig, 1929, Georg Thieme.
6. Mommsen, H.: Die Granula der polymorphkeringen feingekörnten Leukozyten unter normalen und pathologischen Verhältnissen ihre gesetzmässigen Beziehungen zum Ablauf akuter Infectionen, Ztschr. f. d. ges. Exper. Med. **65**: 287, 1929.
7. Ibid.: Die pathologische (toxische) Granulation der feingekörnten Leukozyten, ihre objective Erkennung und praktisch klinische Verwertung, Klin. Wehnschr. **42**: 2420, 1929.
8. Rosenwasser, H., and Rosenthal, N.: The Blood Picture in Otitic Infections, Arch. Otolaryng. **14**: 291, 1931.
9. Kugel, M. A., and Rosenthal, N.: Pathological Changes in Polymorphonuclear Leukocytes During Progress of Infections, Am. J. M. Sc. **183**: 657, 1932.
10. Rosenthal, N., and Sutro, C. J.: The Blood Picture in Pneumonia With Special Reference to Pathological Changes in the Neutrophils, Am. J. Clin. Path. **3**: 181, 1933.
11. Sutro, C. J.: Cytoplasmic Changes in Circulating Leukocytes in Infection, Arch. Int. Med. **51**: 747, 1933.
12. Cooke, W. E., and Ponder, E.: The Polynuclear Count, London, 1927, Griffin & Co., Ltd.
13. Sturgis, C. C., Isaacs, R., and Smith, M.: Limitations of the Liver Treatment in Pernicious Anemia, Trans. Assn. Am. Phys. **43**: 127, 1928.
14. Smithburn, K. C., and Zerfas, L. G.: Inhibitory Action of Infection and Fever on Hematopoietic Response in Case of Pernicious Anemia, Ann. Int. Med. **4**: 108, 1931.
15. Fleming, G. W. T. H.: Liver Treatment of Pernicious Anemia, Brit. M. J. **1**: 638, 1929.
16. Heck, F. J., and Watkins, C. H.: The Neutrophil in Pernicious Anemia, Am. J. Clin. Path. **3**: 263, 1933.
17. Farley, D. L.: Depressed Bone Marrow Function From the Arsphenamines, Am. J. M. Sc. **179**: 214, 1930.
18. Loveman, A. B.: Toxic Granulocytopenia, Purpura Hemorrhagica, and Aplastic Anemia Following Arsphenamines, Ann. Int. Med. **5**: 1238, 1932.
19. Kracke, R. R.: The Experimental Production of Agranulocytosis, Am. J. Clin. Path. **2**: 11, 1932.
20. Selling, L.: Benzol as a Leukotoxin, Bull. Johns Hopkins Hosp. **27**: 83, 1916.
21. Madison, F. W., and Squier, L. L.: Primary Granulocytopenia After Administration of Benzene Chain Derivatives, Proc. Central Society for Clinical Research, Oct., 1933, Abstr. J. A. M. A. **101**: 2076, 1933; The Etiology of Primary Granulocytopenia (Agranulocytic Angina), ibid. **102**: 755, 1934.
22. Watkins, C. H.: The Possible Rôle of Barbiturates and Amidopyrine in Causation of Leukopenic States, Proc. Staff Meet., Mayo Clinic **8**: 713, 1933.
23. Isaacs, R.: Personal Communication.
24. Thayer, W.: Observations on the Blood in Typhoid Fever, Johns Hopkins Hosp. Reports **8**: 487, 1900.
25. Austin, J. H., and Leopold, S. S.: An Extraordinary Polymorphonuclear Leukopenia in Typhoid Fever, J. A. M. A. **66**: 1084, 1916.
26. Schilling, V.: Das Blutbild und seine klinische Verwertung, Fischer, Jena, 1926; translated by R. B. H. Gradwohl, St. Louis, 1929, The C. V. Mosby Co.

ELLIPTICAL HUMAN ERYTHROCYTES*

Observations of Size, Volume and Hemoglobin Content

D. J. Stephens, M.D., and A. J. Tatelbaum, M.D., Rochester, N. Y.

THE occurrence of oval or elliptical erythrocytes in the blood of otherwise apparently normal individuals was first reported by Dresbach[1] in 1904. In a recent comprehensive review of the literature, McCarty[2] has collected reports of 79 instances of this abnormality of the red blood cells. The familial nature of the condition was suspected by Dresbach and has been amply confirmed, in particular, by studies of the families reported by Hunter and Adams[3, 4] and by Cheney.[5] It is the purpose of the present report to present hematologic observations of fifteen members of a family, eight of whom exhibited elliptical erythrocytes.

Rose I., a thirty-five-year-old Italian housewife, was admitted to the Gynecological Service of the Rochester Municipal Hospital in July, 1933, for dilatation and curettage because of excessive menstrual bleeding. There had been no symptoms of anemia or other blood disorder. Her father had died in Italy at the age of forty-five, after a five-year illness, during which his "blood turned to water." The patient had four healthy children. Her mother, sister, and three brothers were living and well.

Physical examination showed a well-developed and nourished woman who did not appear ill. The mucous membranes were of good color. There was no glandular enlargement. The heart and lungs were apparently normal. Blood pressure was 102 mm. Hg systolic, 60 diastolic. There was slight tenderness over the right side of the abdomen and over the liver edge, which was felt just below the costal margin. The spleen was not palpable. On pelvic examination the cervix uteri was found to be enlarged, lacerated and reddened, with widely patent external os. There were small external and internal hemorrhoids.

At the first examination the red blood cells numbered 5,100,000 per c.mm., white blood cells 9,000 per c.mm., hemoglobin 12.8 gm. per 100 c.c. of blood. The differential leucocyte formula was normal but in examining the blood film it was noted that the erythrocytes varied markedly in shape, with a large percentage of oval and elongated forms. Subsequent studies revealed that the variation in shape was constantly present in preparations of the patient's blood and in that of several members of her family. Reticulocyte counts varied between 0.2 and 1.2 per cent. In hypotonic saline, hemolysis of the red blood cells began at 0.38 per cent sodium chloride was complete at 0.26 per cent. In the control, hemolysis began at 0.40 per cent, was complete at 0.28 per cent. Other laboratory procedures, including urinalysis, stool examination, blood Wassermann reaction and cholecystogram, showed no abnormalities. Histologic examination of uterine curettings showed normal endometrium. For a few months some menorrhagia continued and there was occasionally a small amount of bleeding from hemorrhoids. These symptoms were accompanied by a slight decrease in hemoglobin and red blood cells.

*From the Departments of Medicine and of Obstetrics and Gynecology of the University of Rochester School of Medicine and the Strong Memorial and the Rochester Municipal Hospitals.

Received for publication, May 22, 1934.

It was possible to secure blood for study from fifteen members of three generations of the family. Seven of these showed abnormalities in shape of the red blood cells similar to those observed in the patient (Fig. 1). Two of the younger children refused permission for venipuncture; in these, examinations were limited to capillary blood. Carrie R. refused permission to examine the blood of her four children. With the exception of Rose I., all of those examined were without complaints and were apparently healthy; none of them presented evidence of anemia, glandular enlargement, or splenomegaly.

Blood for fresh sealed preparations, fixed smears and reticulocyte counts was obtained from the lobe of the ear. The fixed smears were dried in air and stained with Wright's stain. Reticulocytes were enumerated in "wet" preparations stained with brilliant cresyl blue. The cell counts, hemoglobin estimations and volume of packed red blood cells were determined in venous blood, 5 c.c. of which were drawn into a bottle containing 0.01 gm. of dry potassium oxalate. The blood was thoroughly mixed before sampling. For the

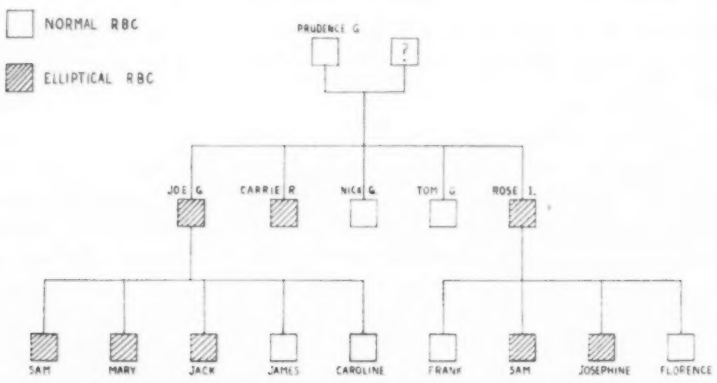


Fig. 1.—Familial distribution of elliptical erythrocytes.

estimation of the reticulocyte counts and the percentage of abnormally shaped erythrocytes in fresh sealed and fixed preparations, 1,000 erythrocytes were enumerated under the oil immersion lens. Hemoglobin was determined with a Sahli hemoglobinometer standardized by oxygen capacity. In order to reduce the error in estimating the number of red blood cells, the average of two counts was taken for each determination. Pipettes and counting chambers certified by the Bureau of Standards were used. Volume of packed red cells was determined by means of the Wintrobe hematocrit tube and corrected for shrinkage due to the anticoagulant. The mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were calculated according to the formulas given by Wintrobe.[6] In estimating the diameter of erythrocytes in fixed, stained smears, 500 cells were measured in two dimensions by means of an ocular micrometer which had been calibrated for microscopic tube length and working conditions.

In the blood of the eight individuals with elliptical erythrocytes, the percentage of the abnormally shaped cells varied from 20 to 56 per cent. They were present in greatest numbers in the blood of Rose I. There was considerable

variation in different films taken at the same time. In general, higher values were encountered in wet than in dry preparations. Although the majority of the "elliptical" cells were oval in outline, a few elongated, rodlike and irregularly shaped cells were observed in each preparation. The relative frequency of oval, elongated and irregularly shaped cells in both wet and dry preparations was approximately 8, 1.5, 1. All types of intermediate forms were encountered so that determinations of frequency of morphologic types were but rough estimates. In the wet preparations the oval and elongated cells were apparently biconcave ellipsoids. The ends of the cells were uniformly blunt and rounded. In general appearance they seemed similar to the poikilocytes commonly seen in the microcytic and macrocytic anemias. Crescent forms and filamentous processes characteristic of the cells in sickle-cell anemia were not seen. In staining characteristics and general morphologic appearance the oval and elongated cells seemed similar to the round cells. In Fig. 2 are shown photo-

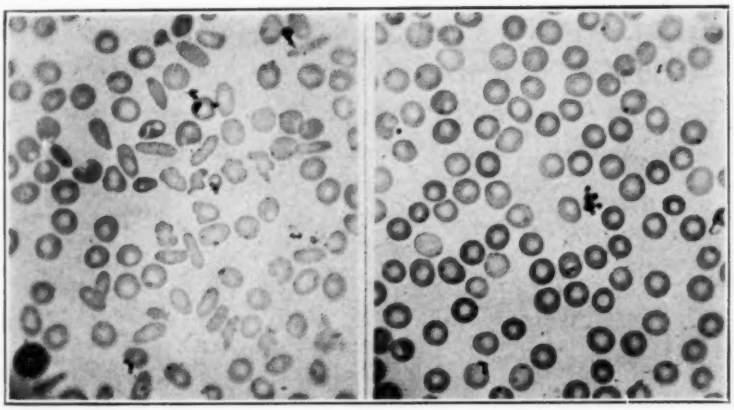


Fig. 2.—Photomicrographs of fixed stained blood films. Left, Rose I. Right, Tom G.

micrographs of fixed, stained smears of the blood of Rose I. and of her brother, Tom, whose red blood cells were apparently normal.

In individuals whose erythrocytes were classed as normal, an occasional oval cell was encountered but in these the degree of ellipticity was slight and no elongated or markedly irregular cells were encountered. The percentage of oval forms in wet and dry preparations of the blood of these was no greater than that frequently observed in other healthy persons, for the most part less than 1 per cent, in no case more than 5 per cent.

Detailed studies were made in the case of Rose I. for further identification of the type of the prevailing abnormality. Several wet preparations were observed over a period of twenty-four hours without change in the degree or frequency of the abnormal forms. The abnormality persisted unchanged after six hours in the ice box and in the incubator at a temperature of 56° C. The anomaly was present to approximately the same extent in wet, sealed preparations of capillary blood, oxalated blood, saline suspensions and in hanging drop

preparations. On three occasions the cells were studied before and after twenty minutes' exposure to carbon dioxide according to the technic described by Hahn and Gillespie.[7] No change was noted in the shape of the cells or in the frequency of the abnormal forms. The appearance of the cells, their behavior under the above conditions, the familial distribution and the lack of significant anemia identified the anomaly as that which has been described as "elliptical human erythrocytes" or "ovalocytosis."

Quantitative hematologic observations are summarized in Table I. In affected individuals, the erythrocytes were uniformly above 5 million per c.mm., but the hemoglobin was not correspondingly increased. In spite of the apparent polycythemia the volume of packed red blood cells was within normal limits, so that in each instance the mean corpuscular volume and mean corpuscular content of hemoglobin were definitely decreased. However, the degree of saturation of corpuscular mass with hemoglobin (MCHC) was not significantly lowered. In those members of the family whose red blood cells were considered normal, corpuscular volume and hemoglobin content were well within the generally accepted normal range. In two of the latter group, the erythrocytes numbered more than 5 million per c.mm., but in these there was a corresponding increase

TABLE I

HEMATOLOGIC OBSERVATIONS OF FIFTEEN MEMBERS OF THE FAMILY OF ROSE I.

SUBJECT	AGE	DATE	ELLIPTICAL CELLS	HEMOGLOBIN GRAMS PER 100 C.C.	RED BLOOD CELLS MILLIONS PER C.MM.	V.P.R.* (COR.) C.C. PER 100 C.C.	M.C.V.*	M.C.H.*	M.C.H.C.*	RETICULOCYTES PER CENT	WHITE BLOOD CELLS PER C.MM.
Rose I.	35	8/10	+	13.05	5.97	40.9	68.4	21.7	31.9		9,200
		8/17	+	12.3	5.91	39.2	66.4	20.8	31.4	1.2	8,850
		2/16	+	11.3	5.46	36.0	65.9	20.7	31.4	0.2	
Sam I.	9	8/17	+	12.75	7.15	38.0	53.1	17.8	33.5	1.0	8,100
Josephine I.	8	8/17	+	12.15	5.51	36.0	65.4	22.1	33.8	1.0	9,250
Joe G.	43	8/10	+	15.5	7.27	46.8	64.3	21.3	33.1	0.8	7,650
		8/24	+	15.0	8.06	46.8	58.1	18.6	32.1	1.2	8,000
Sam G.	17	8/24	+	13.8	6.80	43.1	63.4	20.3	32.0	0.2	9,100
Mary G.	11	8/24	+	13.2	7.98	40.3	50.5	16.5	32.8	0.6	8,500
Jack G.	10	8/24	+	12.6	5.72	38.1	66.6	22.0	33.1	0.8	8,400
Carrie R.	37	9/27	+	15.0	5.36	40.9	76.2	28.0	36.7	1.0	6,800
Average			+	13.3	6.47	40.6	63.5	20.9	32.9	0.8	
Prudence G.	67	9/27	0	15.45	4.94	43.6	88.1	31.2	35.4	0.6	13,500
Frank I.	10	8/17	0	14.85	4.82	42.5	88.1	30.8	35.0	0.6	9,400
Florence I.	5	8/17	0								
James G.	8	8/24	0	12.45	4.33	36.0	83.1	28.8	34.6	0.2	12,500
Caroline G.	7	8/24	0	14.25	4.90			29.1		1.0	19,500
Nick G.	31	8/10	0	18.1	6.05	50.1	83.0	30.0	36.1	0.2	7,700
		3/25	0	17.7	6.40	52.0	81.2	27.7	34.0	0.8	8,000
Tom G.	28	8/10	0	17.8	5.65	49.0	86.8	31.5	36.3	0.4	5,200
		3/25	0	16.6	5.42	47.9	88.4	30.6	34.6	0.6	5,500
Average			0	15.9	5.31	45.9	85.5	30.0	35.1	0.6	

*V. P. R., volume packed red cells.
M. C. V., mean corpuscular volume in cubic microns.
M. C. H., mean corpuscular hemoglobin in micromicrograms.
M. C. H. C., mean corpuscular hemoglobin concentration in per cent.

in hemoglobin and volume of packed red cells. In each instance the number of reticulocytes was within normal limits. There was no increase in the icterus index. Except for a mild neutrophilic leucocytosis in three of the normals, there were no abnormalities in the total white blood cell or differential counts. In fixed smears the platelets appeared to be normal in number and structure.

In examining the wet and fixed blood films of various members of the family, difference in the size of the erythrocytes in the two groups was apparent. Two dimensional measurements of the red blood cells were made in fixed, stained cover slip preparations of the blood of four individuals with elliptical cells and in four subjects of similar age with normal cells. The results of these studies are shown in Table II. In each of the four normals, the figures are in agreement with the values given by Price-Jones[8] and by Wischnewsky[9] for the mean diameter of normal human erythrocytes in fixed, stained smears. In con-

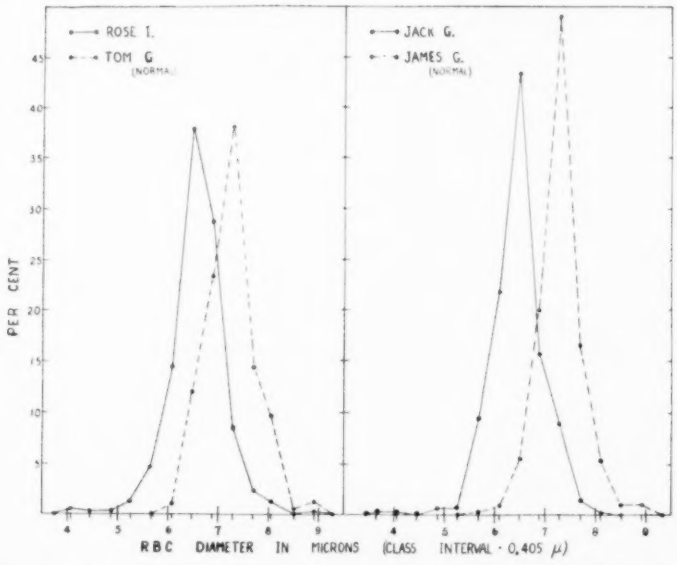


Fig. 3.—Frequency distribution curves of red blood cell diameters in two individuals with elliptical erythrocytes and in two subjects with red blood cells of normal shape.

trast, the round cells of those individuals exhibiting a large number of elliptical cells were consistently and significantly smaller, averaging 6.52 microns in diameter. Frequency distribution curves of round cell diameters of two members of each group are shown in Fig. 3. The calculated average diameter of the elliptical cells was about the same as the observed diameter of the round cells. Measurements of the length and width of the oval and elongated cells were scattered over a wide range, as indicated in the table. However, the majority of the abnormally shaped cells were oval in outline and the variation in the average dimensions of these cells was of approximately the same magnitude as for the round cells. In considering the above results it should be noted that drying and staining causes an appreciable shrinkage of erythrocytes, so that cells in fixed, stained preparations are 1 to 1.5 microns smaller than the same cells in plasma, serum, or isotonic saline.

TABLE II

TWO DIMENSIONAL MEASUREMENTS, IN MICRONS, OF RED BLOOD CELLS OF EIGHT MEMBERS OF THE FAMILY

SUBJECT	ROUND CELLS				ELLIPTICAL CELLS						
	MEAN DIAMETER	STANDARD DEVIATION	COEFFICIENT OF VARIATION	PROBABLE ERROR	UPPER LENGTH	LOWER LENGTH	UPPER WIDTH	LOWER WIDTH	AVERAGE LENGTH	AVERAGE WIDTH	CALCULATED MEAN DIAMETER
Rose I.	6.59	0.554	8.4%	0.0186	12.16	5.67	7.29	1.62	8.15	5.38	6.76
Joe G.	6.73	0.234	3.5%	0.0075	12.16	4.86	7.29	1.62	7.32	5.44	6.38
Jack G.	6.44	0.515	8.1%	0.0163	11.35	6.48	7.29	3.24	8.06	5.29	6.67
Sam I.	6.50	0.404	6.5%	0.0129	9.72	5.67	7.29	4.05	8.72	5.02	6.87
Average	6.56	0.427	6.6%	0.0141	11.30	5.67	7.29	2.63	8.06	5.28	6.67
Nick G.	7.31	0.620	8.4%	0.0188							
Tom G.	7.25	0.510	6.9%	0.0154							
James G.	7.28	0.423	5.8%	0.0129							
Frank I.	7.24	0.546	7.5%	0.0168							
Average	7.27	0.537	7.1%	0.0159							

The calculated mean diameter of elliptical cells is taken as the mean of the average length and the average width.

The nature and etiology of the observed abnormality have been the subject of much discussion. Several observations are on record of studies of bone marrow obtained at autopsy or by sternal puncture.[6, 10, 11, 12, 13] Red blood cell progenitors have uniformly been round in shape, although mature erythrocytes in sections of bone marrow and other tissues have appeared similar in outline to those observed in preparations of circulating blood. Nucleated red blood cells have not been observed in the peripheral circulation. During the present study a large proportion of the reticulocytes observed in the blood of affected individuals were definitely oval in outline but the degree of ellipticity was not marked. No elongated or grossly irregular reticulocytes were seen. The abnormality in shape persists in various isotonic solutions, in surviving cells in hypotonic saline, in the serum of normal individuals and in the presence of the usual anticoagulants. The percentage of abnormally shaped cells in cases reported in the literature has varied from 10 per cent to more than 90 per cent; there are no recorded observations of 100 per cent elliptical cells. In the same patient, considerable variation in frequency and degree of poikilocytosis is encountered in individual preparations taken at the same or different times. In general the anomaly is more marked in wet than in dry preparations. Terry, Hollingsworth and Eugenio[10] cite experiments which indicate that the temperature and the degree of trauma to which the cells are subjected in preparing them for examination may influence their shape, and that the oval cells are heavier and more resistant to hypotonic saline than are the round erythrocytes. The authors and others[2] have been unable to confirm these observations. Two dimensional measurements indicate that the abnormally shaped cells are of approximately the same size as the round cells and that the variability of distribution of cell size is approximately normal. In both wet and fixed preparations the normal and abnormal cells appear to be similar morphologically except for the difference in shape. It is probable that in affected individuals the potentiality for the deformity is possessed by all the mature erythrocytes, perhaps to a lesser degree by the nonnucleated reticulocytes. The factors which determine the observed shape of individual cells have not as yet been clearly defined.

In the authors' cases, each of the affected individuals showed a definite decrease in mean corpuscular volume and in mean corpuscular hemoglobin content. Two dimensional measurements of the erythrocytes in fixed, stained smears indicated that both the round and abnormally shaped cells contributed to the decrease in mean cell volume. The decrease in average cell size was partially, in some instances completely, compensated for by an increase in the red blood cell count so that the average volume of packed red blood cells was approximately normal. Except for a shift toward the smaller cell groups, the frequency distribution curves of cell size was approximately the same in the affected as in the normal individuals (Fig. 3). This may be contrasted with the increase in variability of cell size and the marked broadening and flattening of the frequency distribution curve, which usually accompany the poikilocytosis of the macrocytic and microcytic anemias.[8] A similar increase in variability of cell size may occur in those individuals with elliptical erythrocytes who also present anemia.

In previously reported cases, the red blood cells have been in excess of 5 million per c.mm. in only a few instances. In the majority there have been varying degrees of anemia, with the color index usually below one. One would expect the latter finding to be accompanied by a decrease rather than an increase in the mean corpuscular volume. The available literature contains few observations of the size of elliptical human erythrocytes. In Dresbach's case,[1] average size of the elliptical cells was 10.3 by 4.1 microns; extremes in length were 10.7 and 8.5 microns, in width 4.8 and 3.9 microns. The cells of Bishop's patient[14] were measured in dry films; the round cells averaged 8.5 microns in diameter, the elliptical cells varied in length from 17 to 8.2 microns, in width from 8.0 to 3.2, averaging 13 by 5 microns in size. Sydenstricker[15] recorded variations of from 13.0 to 8.5 microns in length, 4 to 6 microns in width, with the round cells 8 to 8.5 microns in diameter. Huck and Bigalow[16] measured the cells of one of their patients in both wet and dry preparations. In the former the round cells varied from 6 to 11 microns in diameter, in the latter from 5 to 9 microns; in this patient the red blood cell count was well over 5 million per c.mm. and the color index was about 0.8. In a patient with anemia and color index less than one, Hunter and Adams[3] found the round cells to have a mean diameter of 9 microns. In two of McCarty's patients, both with anemia, the volume index was 1.23 and 1.50.[2] In the latter, the mean diameter of the round cells measured in wet preparations was only 8.0 microns, in spite of the high volume index; in this patient the oval cells measured 13.3 by 5.78 by 2.89 microns. The variations in recorded observations are difficult to interpret because of failure in some instances to indicate the method used or the conditions under which the cells were measured. Observations of additional cases under controlled conditions must be made before it can be said with certainty that definite abnormalities in size and hemoglobin content are characteristic of elliptical human erythrocytes.

SUMMARY

1. Hematologic studies are presented of fifteen members of an Italian family, eight of whom exhibited elliptical erythrocytes.

2. In each of the affected individuals, there was an increase in the number of red blood cells per cubic millimeter with definite decrease in mean corpuscular volume and mean corpuscular hemoglobin. In general the number of red blood cells was inversely proportional to the mean cell volume and hemoglobin content.

3. Two dimensional measurements of cells in fixed, stained smears indicated that the decrease in size involved the round cells and the abnormally shaped cells to approximately the same extent, without increase in the variability of distribution of cell size.

4. In the unaffected members of the family the erythrocytes were normal in shape, size, volume, and hemoglobin content.

REFERENCES

1. Dresbach, M.: Elliptical Human Red Blood Corpuscles, Science **19**: 469, 1904.
2. McCarty, S. H.: Elliptical Red Blood Cells in Man, J. Lab. & Clin. Med. **19**: 612, 1934.

3. Hunter, W. C., and Adams, R. B.: Hematologic Study of Three Generations of a White Family Showing Elliptical Erythrocytes, Ann. Int. Med. **2**: 1162, 1929.
4. Hunter, W. C.: A Further Study of a White Family Showing Elliptical Erythrocytes, Ann. Int. Med. **6**: 775, 1932.
5. Cheney, G.: Elliptical Human Erythrocytes, J. A. M. A. **98**: 878, 1932.
6. Wintrobe, M. M.: The Size and Hemoglobin Content of the Erythrocyte, J. Lab. & Clin. Med. **17**: 899, 1932.
7. Hahn, E. V., and Gillespie, E. B.: Sickle Cell Anemia, Arch. Int. Med. **39**: 233, 1927.
8. Price-Jones, C.: Red Blood Cell Diameter, Oxford University Press, London, 1933.
9. Wischnewsky, W. G.: Quoted by Price-Jones.
10. Terry, M. C., Hallingsworth, E. W., and Eugenio, V.: Elliptical Human Erythrocytes, Arch. Path. **13**: 193, 1932.
11. Bernhardt, H.: Ovalocytose der Erythrozyten als Anomalie, Deutsche med. Wchnschr. **54**: 987, 1928.
12. Lawrence, J. S.: Human Elliptical Erythrocytes, Am. J. M. Sc. **181**: 240, 1931.
13. Roth, O., and Jung, E.: Zur Kenntnis der Ovalocytose, Folia Haemat. **44**: 549, 1931.
14. Bishop, F. W.: Elliptical Human Erythrocytes, Arch. Int. Med. **14**: 388, 1914.
15. Sydenstricker, V. P.: Elliptical Human Erythrocytes, J. A. M. A. **81**: 113, 1923.
16. Huck, J. G., and Bigalow, R. M.: Poikylocytosis in Otherwise Normal Human Blood, Bull. Johns Hopkins Hosp. **34**: 390, 1923.

THE COLLOIDAL GOLD REACTION IN 500 CASES OF NEUROSYPHILIS*

William C. Menninger, M.D., Topeka, Kan., and
Leon Bromberg, M.D., Chicago, Ill.

THE following brief report concerns the colloidal gold reaction in 500 cases of neurosyphilis, with particular attention to the variations in the curve in the different clinical types of the disease. The diagnosis in every instance was based primarily on the clinical picture, and was supported by a positive cerebrospinal fluid Wassermann test in all cases. The 500 patients were unselected and many had had varying amounts of antisyphilitic treatment before coming to the clinic.

The colloidal gold reaction in neurosyphilis has been the subject of a great many investigations, both with regard to its diagnostic and prognostic value. The most recent comprehensive study was made by Novy[1] who analyzed the colloidal gold reaction in the cerebrospinal fluids of 500 cases of neurosyphilis. As a control the author used 200 cases of systemic syphilis without central nervous system involvement. Novy concluded: that syphilis without central nervous system invasion may frequently produce some change in the colloidal gold reaction; that no one type of neurosyphilis presents a constant curve; that first zone curves, even when pronounced, do not necessarily indicate general paralysis; that a middle zone curve is not of diagnostic significance since it occurs frequently in all types of neurosyphilis; and that there is not sufficient uniformity in the changes in general to merit prognostic significance.

The results of the present investigation are shown in Table I. The colloidal gold tests were all carried out in the same laboratory according to the standard technic, using a gold sol prepared by the method of Levine,[2] the

*Received for publication, May 28, 1934.

director of the laboratory. For convenience, we have grouped the curves into five classes: (1) a low first zone curve in which the change was limited to the first zone of the curve, and maximum color change did not exceed the value indicated by the number "3"; (2) a strong first zone reaction, in which the maximum was "4" in at least one tube; (3) the so-called "paretic" reaction, in which the change was in the first zone, and reached a maximum of "5," usually in two or more tubes; (4) a low second zone curve, in which the change was limited to the second zone and the maximum color change was "3" or less; (5) a strong second zone reaction with the maximum color change reaching "4" or "5."

TABLE I

COLLOIDAL GOLD REACTION	PARESIS		TABO-PARESIS		MENINGO-VASCULAR NEUROSYPH-ILIS		TABES DORSALIS		ASYMP-TOMATIC NEURO-SYPHILIS	
	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT
Negative	0	0	0	0	1	7.7	4	3.0	14	4.7
(1) Low zone I	3	7.9	2	10.5	2	15.4	31	23.3	53	17.8
(2) Strong zone I	3	7.9	3	15.8	5	38.4	25	18.5	65	21.9
(3) "Paretic"	27	71.1	11	57.8	4	30.8	52	39.0	86	28.9
(4) Low zone II	0	0	2	10.5	0	0	8	6.1	43	14.5
(5) Strong zone II	3	7.9	0	0	0	0	11	8.3	33	11.1
Not done	2	5.2	1	5.2	1	7.7	2	1.5	3	1.0
Totals	38	100.0	19	100.0	13	100.0	133	100.0	297	100.0

General Paresis.—From Table I it is evident that 71.1 per cent of the 38 cases of paresis presented the maximum first zone reaction, often regarded in the past as specific for this type of neurosyphilis. Only 65 per cent of the 100 cases of general paralysis reported by Novy[1] showed the typical maximum first zone curve.

Taboparesis.—As expected, this group showed the next highest percentage (57.8 per cent) of cases with the maximum first zone curve, as compared with 32 per cent in Novy's series. In our cases only two (10.5 per cent) showed a change limited to the second zone and, in both instances, the changes were slight.

Meningovascular Neurosyphilis.—This series of only 13 cases was too small to allow definite conclusions. All fluids in this series showed a deviation of the colloidal gold curve in the first zone.

Tabes Dorsalis.—The significant finding in this group of 133 cases was the large percentage (39 per cent) with a maximum first zone, or so-called "paretic" type of curve. (Only 11 per cent of Novy's 100 cases of tabes dorsalis showed this type of reaction.) Four of our cases showed no change in the colloidal gold reaction despite very definite clinical evidence of tabes, as well as a positive cerebrospinal fluid Wassermann test.

Asymptomatic Neurosyphilis.—In this large series of 297 cases, we found 28.9 per cent showing the so-called "paretic" type of curve, and 68.6 per cent showing some change limited to the first zone as compared to 25.6 per cent showing changes limited to the second zone. Fourteen cases (4.7 per cent) showed no change in the colloidal gold reaction.

CONCLUSION

1. An analysis of the colloidal gold reaction in the cerebrospinal fluids of 500 patients suffering from neurosyphilis showed no constant correspondence between the pattern and degree of precipitation and the type of central nervous system involvement.

2. The colloidal gold curve, while of considerable confirmatory value in the presence of clinical signs of general paresis, is in itself not diagnostic.

REFERENCES

1. Novy, F. G.: The Significance of the Colloidal Gold Reaction as a Diagnostic and Prognostic Aid in Neurosyphilis, Am. J. M. Sc. **183:** 401, 1932.
2. Levine, B. S.: Making Gold Sol for Spinal Fluid Tests, Am. J. Syph. **16:** 103, 1932.

A COMPARISON OF VARIOUS CONCENTRATIONS OF SODIUM OXALATE SOLUTION WITH HEPARIN FOR THE DETERMINATION OF PACKED CELL VOLUME*

ORVILLE S. WALTERS, PH.D., ST. LOUIS, MO.,
AND JAMES W. MAY, LAWRENCE, KAN.

THE utilization of standard centrifuge tubes for the routine determination of packed cell volume appears to have originated with Keith, Rowntree and Geraghty (1915) in connection with blood volume studies. They employed dry oxalate as an anticoagulant. The use of an isotonic oxalate solution to prevent shrinkage of cells was suggested by Hooper, Smith, Belt, and Whipple (1920) who introduced 1.6 per cent sodium oxalate solution for this purpose.

The choice of this concentration of sodium oxalate was based, by these investigators, both on physicochemical relations and biologic tests. In the first instance, they ascertained from chemical tables that a 1.6 or a 1.7 per cent solution of sodium oxalate would be approximately isotonic with a 0.95 per cent solution of sodium chloride if the dissociations of sodium and potassium oxalates were not far different.

The biologic tests consisted of three experiments. Ten cubic centimeters of defibrinated dog's blood were placed in each of eight tubes. Seven of these contained 2 c.c. quantities of sodium oxalate in concentrations varying from 1.0 to 1.6 per cent. The eighth tube contained no anticoagulant. Two such sets of tubes were centrifuged at 3,000 r.p.m. for one-half hour in the first experiment. The second experiment was identical except that blood from a different dog was used. Comparison of the single control tube with the others in each of these four series showed that the packed cell volume in the tube containing 1.6 per cent oxalate solution was closest to control value.

In the third experiment, blood from a dog in peptone shock was employed. Four tubes of this blood were centrifuged. Three contained 2 c.c.

*From the Department of Physiology, University of Kansas.
Received for publication, June 18, 1934.

respectively of 1.0 per cent, 1.4 per cent, and 1.7 per cent sodium oxalate solution, while the fourth contained no anticoagulant. In this experiment, the 1.4 per cent solution was hypotonic and the 1.7 per cent hypertonic. By interpolation, the point of isotonicity was considered to be 1.5 per cent.

From these tests and the physicochemical data, a 1.6 per cent solution was adopted by Hooper and others as being approximately isotonic with dog's blood. This concentration was subsequently accepted by Haden (1923) and various other investigators for use on human blood.

The objections are raised by Van Allen (1925) to the work described above that in the defibrination of blood, corpuscles would necessarily be lost and also that the average size of erythrocytes is reduced in peptone shock. Either factor would tend to suggest the 1.6 per cent concentration as being too high. Van Allen states that a 1.5 per cent solution of oxalate is required for ascertaining the packed cell volume of rabbit's blood by the Hooper method. With his capillary hematocrit, however, Van Allen recommends 1.3 per cent oxalate as being in best osmotic agreement with rabbit's blood.

Smith and Prest (1932) adopted 1.3 per cent sodium oxalate in their studies on human blood after comparison of various concentrations with the Van Allen hematocrit. They report that this percentage gives values very close to those for whole blood.

In the experiments conducted by Haden (1930) on human blood with 12 c.c. centrifuge tubes, 1.4 per cent oxalate gave packed cell volumes closer to the values obtained with hirudin and heparin than any other concentration. This per cent of oxalate has since been generally employed for routine use with human blood.

The present study was made to determine by an adequate series of comparisons the concentration of sodium oxalate which yields packed cell volume values for dog's blood agreeing most perfectly with those in which heparin is employed as the anticoagulant.

METHODS

Two healthy 35 kg. dogs were used to furnish the necessary blood. They were alternated to avoid any pronounced anemia. In each experiment, a 100 c.c. quantity of blood was withdrawn from the external jugular vein into a large syringe containing 1 c.c. of 0.35 per cent heparin in 0.9 per cent sodium chloride solution, an amount alone sufficient to prevent coagulation. The blood and heparin were thoroughly mixed in the syringe, then exactly 40 c.c. were run into a graduate cylinder containing 8 c.c. of the sodium oxalate solution to be compared. This was mixed carefully by inversion and immediately divided equally among eight 12 c.c. graduated centrifuge tubes.

The remainder of the heparinized blood was divided among a second series of 12 c.c. tubes. The two sets of tubes (16 in all) were then centrifuged for one hour, removed and read, and returned for additional rotation until the volume remained constant. After correction for the oxalate added, the percentages were calculated and recorded. All readings were made by the same individual.

Two centrifuges were used in the experiments, an International 1SB and an International 1C. Each has a working radius of 9 cm., but the former was run at a speed of 3,600 r.p.m. while the latter was rotated at 2,800 r.p.m.

In all, 50 experiments were conducted. A total of 800 tubes were centrifuged and read, less a few which broke in the machines. The concentrations of sodium oxalate compared were 1.35 per cent, 1.4 per cent, 1.5 per cent, 1.6 per cent and 1.7 per cent. The accuracy of the solutions was checked by a titrimetric method.

RESULTS

The average figures for the experiments using 1.4, 1.5, and 1.6 per cent oxalate solutions are shown in Tables I, II, and III. In Table IV, the comparative data for all concentrations of oxalate compared are summarized. From this table it is apparent that 1.35 and 1.4 per cent solutions of oxalate are definitely hypotonic, since the packed cell volumes are greater than those with heparin alone. The 1.6 per cent and 1.7 per cent solutions are definitely hypertonic, giving packed cell volumes lower than heparin alone. The packed

TABLE I

COMPARATIVE PACKED CELL VOLUMES

Heparin and 1.4 Per Cent Sodium Oxalate Solution

EXPERIMENT	HEPARIN-OXALATE		HEPARIN ONLY	
	PACKED CELL VOLUME	STANDARD DEVIATION	PACKED CELL VOLUME	STANDARD DEVIATION
16	45.1	0.755	44.6	0.464
17	37.7	0.850	38.5	0.529
18	42.5	1.030	41.6	0.552
19	38.1	0.775	37.9	0.458
20	36.9	0.446	36.7	0.847
21	36.3	0.421	37.2	0.815
22	31.5	0.335	31.4	0.731
23	41.9	0.187	38.0	0.423
24	36.0	0.595	36.2	0.619
25	34.5	0.414	33.6	0.432
Mean	38.05	0.5808	37.57	0.5870

TABLE II

COMPARATIVE PACKED CELL VOLUMES

Heparin and 1.5 Per Cent Sodium Oxalate Solution

EXPERIMENT	HEPARIN-OXALATE		HEPARIN ONLY	
	PACKED CELL VOLUME	STANDARD DEVIATION	PACKED CELL VOLUME	STANDARD DEVIATION
26	34.8	0.609	34.5	0.581
27	39.3	0.704	39.8	0.470
28	38.5	0.658	38.9	0.501
29	34.2	0.328	33.7	0.595
30	34.8	0.494	34.7	0.261
31	35.4	0.863	36.2	0.501
32	36.6	0.480	35.8	0.335
33	35.4	0.664	35.5	0.446
34	34.9	0.476	34.9	0.426
35	28.9	0.577	28.5	0.414
Mean	35.28	0.5853	35.25	0.4530

cell volume values obtained with 1.5 per cent sodium oxalate solution, however, are practically identical with those in which heparin alone was employed as the anticoagulant.

Error of the Method.—The coefficient of variation derived from simultaneous determinations on the same sample of blood has been used as a measure of the error inherent in the method. This apparently has not been ascertained for the standard tube method, although Wintrobe (1930) found variations no greater than 0.5 per cent with his 3 mm. bore hematocrit tubes.

TABLE III

COMPARATIVE PACKED CELL VOLUMES

Heparin and 1.6 Per Cent Sodium Oxalate Solution

EXPERIMENT	HEPARIN-OXALATE		HEPARIN ONLY	
	PACKED CELL VOLUME	STANDARD DEVIATION	PACKED CELL VOLUME	STANDARD DEVIATION
36	32.6	1.082	32.5	0.466
37	37.9	0.476	39.0	0.576
38	35.4	0.596	36.2	0.550
39	32.1	0.385	32.3	0.256
40	32.5	1.045	32.6	0.737
41	29.5	0.289	30.3	0.570
42	46.3	0.570	46.4	0.514
43	46.4	0.765	47.8	0.975
44	44.8	0.926	44.9	0.499
45	43.3	0.280	44.6	0.699
Mean	38.08	0.6414	38.66	0.5842

TABLE IV

SUMMARY OF ALL EXPERIMENTS SHOWING COMPARISON OF VARIOUS SODIUM OXALATE SOLUTIONS WITH HEPARIN

NO. EXPER.	CONC. OF OXALATE SOLUTION	HEPARIN-OXALATE			HEPARIN ONLY			DIFFERENCE OF OXALATE (PACKED CELL VOLUME)
		NO. OF TUBES	AVER. PACKED CELL VOLUME	AVER. STANDARD DEVIATION	NO. OF TUBES	AVER. PACKED CELL VOLUME	AVER. STANDARD DEVIATION	
15	1.35	120	43.86	0.7687	120	43.19	0.5735	+0.67
10	1.40	80	38.05	0.5808	77	37.57	0.5870	+0.48
10	1.50	80	35.28	0.5853	76	35.25	0.4530	+0.03
10	1.60	79	38.08	0.6414	80	38.66	0.5842	−0.58
5	1.70	40	43.26	0.8136	40	43.80	0.7938	−0.54
Mean			39.77	0.6789		39.63	0.5763	

TABLE V

EFFECT OF QUANTITY OF BLOOD USED

QUANTITY OF BLOOD	HEPARIN-OXALATE				HEPARIN			
	NO. EXPER.	NO. TUBES	AVER. STAND. DEV. (C.C.)	AVER. COEFF. OF VAR. (PER CENT)	NO. EXPER.	NO. TUBES	AVER. STAND. DEV. (C.C.)	AVER. COEFF. OF VAR. (PER CENT)
10.0 c.c.	6	48	0.3997	1.007	--	---	-----	----
7.5 c.c.	--	---	-----	----	44	348	0.5670	1.428
5.0 c.c.	44	351	0.7170	1.806	6	45	0.6228	1.569

The limit of error thus determined for the standard tube method in the present study averages 0.679 c.c. (standard deviation) or 1.71 per cent (coefficient of variation) for heparin-oxalate tubes and 0.576 c.c. or 1.45 per cent for heparin alone. As this difference in variation became apparent in the course of the study, the procedure was varied slightly to ascertain the effect of different amounts of blood, since a larger quantity was being used in the heparin series (7.5 c.c. in each tube) than in the oxalate series (5 c.c. in each tube).

The results are shown in Table V. A much smaller error, approximately 1 per cent, was found when the amount of blood in the oxalate tubes was increased to 10 c.c. Likewise, the limit of error of the heparin tubes increased from 1.43 to 1.57 per cent when a smaller quantity of blood was employed.

As a further modification in the method, both 15 c.c. and 12 c.c. tubes were used. The comparative data are given in Table VI. It will be seen that the error is slightly greater in the 15 c.c. than in the 12 c.c. tubes.

TABLE VI

EFFECT OF SIZE OF TUBE USED

SIZE OF TUBE	NO. EXPER.	HEPARIN-OXALATE (5 C.C. BLOOD)			HEPARIN (7.5 C.C. BLOOD)		
		NO. TUBES	AVER. STAND. DEV.	COEFF. OF VARIATION (PER CENT)	NO. TUBES	AVER. STAND. DEV.	COEFF. OF VARIATION (PER CENT)
15 c.c.	20	160	0.7384	1.860	159	0.6059	1.526
12 c.c.	24	191	0.7010	1.766	189	0.5473	1.379

A careful check was kept of the number of tubes in which packing was complete at the end of one hour. Of the nearly 800 tubes, 97.5 per cent showed no change in volume after one hour of rotation.

SUMMARY

1. A series of 800 comparative packed cell volume determinations made by the standard centrifuge tube method on fifty 100 c.c. samples of dog's blood demonstrates that a 1.5 per cent solution of sodium oxalate gives values practically identical with those obtained with heparin.

2. The minimum error found for the standard tube method was approximately 1 per cent, obtained when 10 c.c. quantities of blood were employed. The error is proportionately greater when smaller quantities of blood are used. It is slightly less with 12 c.c. than with 15 c.c. tubes.

3. Packing was complete after one hour of rotation in 97.5 per cent of cases.

REFERENCES

Haden, R. L.: Accurate Criteria for Differentiating Anemias, Arch. Int. Med. **31**: 766, 1923.

Haden, R. L.: The Technic of Determination of the Relative Mass, the Individual Cell Volume, and the Volume Index of the Erythrocytes of Man, J. LAB. & CLIN. MED. **15**: 736, 1930.

Hooper, C. W., Smith, H. P., Belt, A. E., and Whipple, G. H.: Blood Volume Studies. I. Experimental Control of a Dye Blood Volume Method, Am. J. Physiol. **51**: 205, 1920.

Keith, N. M., Rowntree, L. G., and Geraghty, J. T.: A Method for the Determination of Plasma and Blood Volume, Arch. Int. Med. **16**: 547, 1915.
Smith, C., and Prest, M.: Further Observations on the Normal Variations in Erythrocyte Values in Women, Am. J. Physiol. **99**: 562, 1932.
Van Allen, C. M.: An Hematocrit Method, J. Lab. & Clin. Med. **10**: 1027, 1925.
Wintrobe, M. M.: Blood of Normal Young Women Residing in a Sub-Tropical Climate, Arch. Int. Med. **45**: 287, 1930.

THE OCCURRENCE OF EGGS OF *HETERODERA RADICICOLA* IN HUMAN FECES*

Alvin E. Keller, M.D., Nashville, Tenn.

IN 1919 during the course of a hookworm study among troops at Camp Travis, Texas, Kofoid and White demonstrated nematode eggs of unusual size which were found in 429 cases from approximately 140,000 examinations of feces from soldiers. These eggs had an average dimension of 95 by 40.2 micra but varied markedly in size, the variation in length being from 68 to 133 micra and in width from 33 to 44 micra. They tentatively assigned the name of *Oxyuria incognita* to this egg, and reported their findings in the *Journal of the American Medical Association*.

In 1923 Sandground reported that the egg which was found by Kofoid and White was probably that of *Heterodera radicicola*, a common nonpathogenic nematode which occurs widely in a large number of root vegetables, such as radishes, celery, turnips, and potatoes.

Sandground was able to show that when noninfested individuals ingested parasitized roots of beans along with the usual food it was possible to recover numerous Heterodera eggs the following day. Sandground does not state in his experimental observations whether worms or only eggs were swallowed with parasitized roots but he infers that the eggs rather than adult worms are ingested with food. These eggs, after they were passed from the individuals, were brown in color and were in all stages of development. They were usually symmetrically ellipsoidal but were frequently kidney shaped. They contained small fat globules. Sandground also stated that in the embryo the esophagus and esophageal bulb were similar to the same structures seen in the larvae of *Heterodera radicicola*. Since that time the eggs which were identified by Kofoid and White have been considered to be those of *Heterodera radicicola*.

In the course of a hookworm investigation conducted in Mississippi, similar eggs were found in the specimens of feces examined from the white and negro population of that state. These eggs were demonstrated in 34 specimens from 44,380 white persons and in 5 specimens from 6,441 negroes. The eggs varied in length from 84 to 96 micra and in width from 30 to 42 micra. They were ellipsoidal in shape but usually had a concavity on one side. Most of the eggs seen had a thin double contour. They were light brown in color and the ma-

*From the Department of Preventive Medicine and Public Health, Vanderbilt University.
Received for publication, May 31, 1934.

terial inside the egg was more or less amorphous or globular in character with large clear hyaline-like polar bodies. In some eggs there were two polar bodies while in others only one such structure could be demonstrated. Occasionally the remains of an embryo could be identified. Upon investigation of the literature and with the assistance of Doctor Asa C. Chandler of the Rice Institute, Houston, Texas, these eggs were identified as those of *Heterodera radicicola*. Several drawings of these eggs as they were seen in microscopic slide preparations are shown in Fig. 1.

The 39 cases discovered in this series were distributed in 16 counties in Mississippi, 10 cases coming from 8 counties in northern Mississippi and 29 from 8 counties in southern Mississippi. The majority of the cases in southern Mississippi were from 2 counties. Twenty-eight of the 39 cases were found among children fourteen years of age or less. These eggs were found both by the Stoll dilution egg-counting technic and the brine flotation method of examination. In the specimens examined by the dilution egg-counting technic,

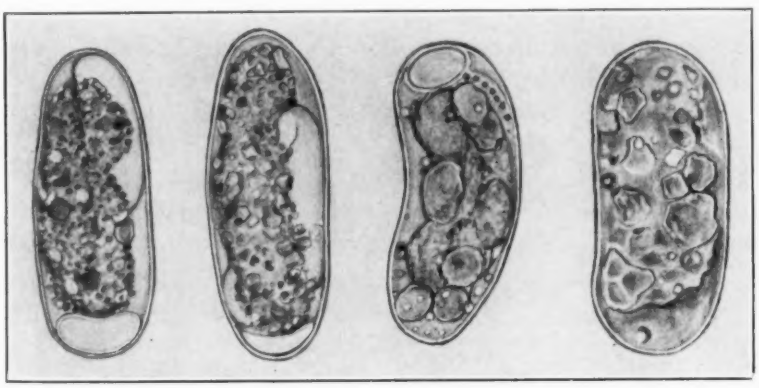


Fig. 1.—Eggs of *Heterodera radicicola*.

there was an average of 700 eggs per gram of feces, indicating a light intensity of infestation. In the specimens found positive by the brine flotation technic, only a few eggs were found in most cases in that portion of the specimen examined microscopically.

The finding of the eggs of *Heterodera radicicola* in human feces represents, according to Chandler, the accidental ingestion of these eggs with raw vegetables, the roots of which have become contaminated with this free living nematode. There is also the possibility that in the collection of specimens of human feces which have been deposited on the surface of the soil and examined for human helminth eggs the eggs of free living nematodes may be included from the soil contamination. This is unlikely due to the fact that adult *Heterodera radicicola* are usually found clinging to roots and vegetables which lie deeper in the soil and not on the surface, making it unlikely that these eggs would be included in specimens of human feces obtained after coming in contact with the soil. This possibility does emphasize, however, that in the collection of specimens of feces care should be taken to prevent the inclusion of soil with the specimen.

Recent investigations in Ireland concerning the relationship of this parasite to soil indicate that it is most frequently found in areas where the soil is sandy. The distribution of the cases in the present study also shows this relationship to sandy soil, as a majority of the specimens were from southern Mississippi, especially in the counties where the percentage of sand in the soil varies from 56 to 88 per cent.

The presence of eggs of *Heterodera radicicola* in the feces causes difficulty in diagnosis and may result in an error due to the fact that microscopists may not be familiar with the appearance of this nematode egg and may report the presence of some of the common eggs such as hookworm or unfertilized ascaris eggs which resemble eggs of *Heterodera radicicola* in some respects. The failure to recognize the eggs of *Heterodera radicicola* may not only result in an error in diagnosis but also may lead to the unnecessary administration of anthelmintics.

SUMMARY

1. During the course of a hookworm investigation in Mississippi the eggs of *Heterodera radicicola* were found in 34 specimens of feces out of 44,380 specimens examined from white persons and in five instances out of 6,441 specimens obtained from negroes.

2. These eggs were found in specimens of feces examined by both the Stoll dilution egg-counting technic and the brine flotation method.

3. Only a few eggs were found in that portion of the specimen examined.

4. Of the 39 cases 28 were found among children under fourteen years of age. Twenty-nine cases were from southern Mississippi and ten were from northern Mississippi.

5. The finding of the eggs of *Heterodera radicicola* in human feces represents the accidental ingestion of these eggs with raw vegetables, the roots of which have become contaminated with this free living nematode.

6. The presence of eggs of *Heterodera radicicola* may result in an error in diagnosis due to their similarity in some respects to unfertilized ascaris and hookworm eggs and result in the unnecessary administration of anthelmintics.

REFERENCES

Carroll, J. A.: A Study of the Potato Eelworm (*Heterodera schachtii*) in the Irish Free State, J. Helminth. **11:** 137, 1933.

Chandler, A. C.: Hookworm Disease, New York, 1929, The Macmillan Company, p. 313.

Kofoid, Charles A., and White, A. W.: A New Nematode Infection of Man, J. A. M. A. **72:** 567, 1919.

Sandground, J. H.: *Oxyuris incognita* or *Heterodera radicicola?* J. Parasitol. **10:** 92, 1923.

THE VIRUS OF LYMPHOGRANULOMA INGUINALE*

Its Cultivation, Its Antigenic Value as a Vaccine and Also in the Production of an Antiserum

Joseph T. Tamura, M.Sc., Cincinnati, Ohio

A REVIEW of the literature reveals that all attempts to cultivate the etiologic agent of lymphogranuloma inguinale have been failures. We have recently[1] succeeded in doing so by utilizing the medium devised by Maitland, Laing, and Lyth[2] for the cultivation of Vaccinia virus. This medium is made by placing sterile bits of rabbit tissue, kidney, liver, or spleen, in Berkefeld-filtered Tyrode's solution. We have used guinea pig tissue in place of rabbit.

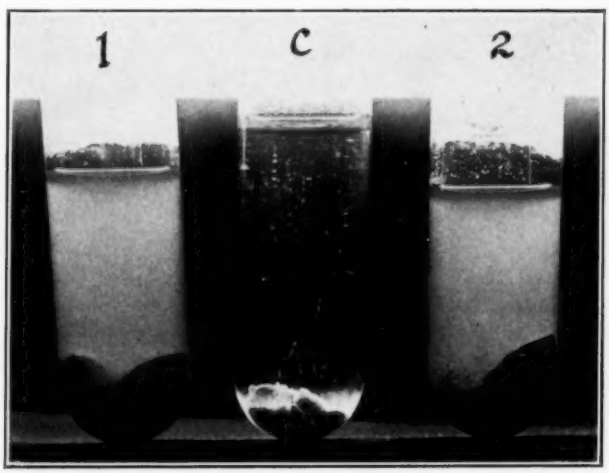


Fig. 1.—Cultivation of lymphogranuloma inguinale virus. *1*, fourteenth subculture from Case C.R. *C*, control. *2*, twelfth subculture from Case H.S., five days' incubation.

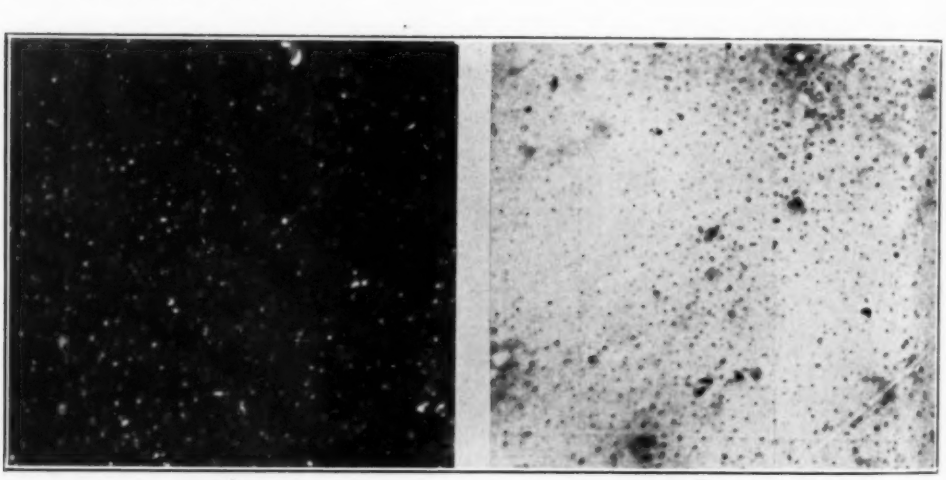
The inoculum, pus, is obtained from a fluctuant inguinal node that has not been exposed to external contamination. This pus is found to be bacteriologically sterile. It is diluted 1:5 with sterile saline solution. When from 0.02 to 0.03 c.c. of the diluted pus is planted in 7 c.c. of Tyrode's solution, containing a piece of guinea pig kidney or liver and incubated at 37.5° C., aerobically a peculiar cloudiness appears throughout the supernatant fluid in thirty-six to forty-eight hours. Control tubes of the medium, incubated in the same manner, remain perfectly clear. This cloudiness is transmissible from one subculture to the next. Fig. 1 illustrates this peculiar formation of cloudiness in the media. In our work the cloudiness was carried through 24, 14, and 12 subcultures when the procedures were discontinued.

*From the Department of Bacteriology and Hygiene, University of Cincinnati College of Medicine, and the Cincinnati General Hospital.

Received for publication, June 2, 1934.

In our experience, as in that of others, cultures made from bubonic pus when planted on a wide variety of bacteriologic media and grown aerobically, at partial oxygen tension, or anaerobically, remain free of visible growth. The cloudiness in the tissue-Tyrode medium remains for twelve- to fourteen-day incubation periods, after which the cloudiness seems to precipitate out and the supernatant fluid becomes clear and slightly straw colored. When this clear supernatant fluid, which has been cloudy once, is subcultured, it no longer produces cloudiness.

When the cloudy supernatant fluid is examined or cultured nothing resembling ordinary bacteria is to be found. Attempts to see the etiologic agent in cloudy fluid have failed with the exception that peculiar granules are brought out by Giemsa's stain and by eosin-Giemsa method originated by Hosokawa[3] for the staining of a variety of viruses. Hosokawa's method of staining viruses, which we used, is as follows:



A. *B.*

Fig. 2.—Cloudy supernatant culture fluid stained with Hosokawa's method, mounted in hyrax. *A*, Dark-field, reduced from a photomicrograph of 1500 diameters. *B*, Direct illumination, reduced from a photomicrograph of 1100 diameters.

1. *Smearing:* Loopful cloudy supernatant fluid is smeared as thin as possible on a perfectly clean slide by means of drawing it lengthwise by a second slide, as in blood smear preparation. The smear is air dried.

2. *Fixation:* The smeared film, after thorough drying in air, is fixed for two minutes in the fixing solution, then washed in water.

The fixing solution:

Methyl alcohol	100.0 c.c.
Formalin (original solution)	5.0 c.c.
Glacial acetic acid	1.0 c.c.

3. *Treating with eosin:* Flood the fixed slide with 1 per cent eosin solution and heat it over a flame for from one-half to one minute until it just steams, as in the case of staining acid-fast bacilli. Wash with water carefully.

4. *Giemsa staining:* Three drops of stock Giemsa in 2 c.c. distilled water is used. Hosokawa solution gave better results. This solution is prepared as follows:

Eosin methylene blue	2.0	gm.
Azure 1	0.4	gm.
Crystal violet	0.025	gm.

Dissolve in 250 c.c. of equal parts of methyl alcohol and glycerin; filter through paper.

One c.c. of the stock solution is diluted in 50 c.c. of distilled water just before using. Fixed and eosin-treated slides are stained from fifty to seventy minutes at room temperature, are washed in water and dried in the air.

It is found that not all of the bubonic pus from the patients produce cloudiness of the medium. When pus from patients that fails to produce cloudiness in media is used to prepare Frei antigen, this antigen fails to give positive skin reactions in proved cases. Subcultures from a 1:10,000 dilution of pus failed to produce cloudiness, but from a 1:1,000 dilution yielded growth. When diluted pus or the cloudy supernatant fluid in culture is passed through a

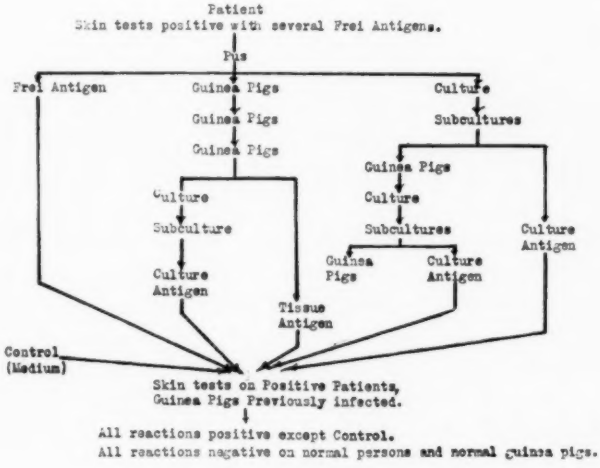


Chart 1.

Berkefeld "N" filter the filtrate will produce cloudiness on subculture. When 1 c.c. of the cloudy supernatant fluid is inoculated into the groin of a guinea pig, a lymphadenitis is produced in from two to three days. The glands gradually increase in diameter and after ten to twelve days will become conglomerate masses of glands and may measure 15 mm. or more in diameter. The overlying skin becomes adherent to the affected nodes, after which they tend to disappear spontaneously. The skin sensitivity of the animals will develop in from three to four weeks. This is comparable to that which one can accomplish by direct inoculation with human pus. Such culture inoculations were successful when the fourth, sixth, and eighth subcultures were used. The etiologic agent has been passed from culture to guinea pig, then from guinea pig to the medium, from this culture to a subculture and then back to the guinea pig. Chart 1 illustrates the complete cycle of animal inoculation.

The cloudy supernatant fluid from a subculture was heated to 60° C. for two hours on one day, and at the same temperature for one hour on the fol-

lowing day, as in the well-known method of preparing Frei's antigen from pus. When such heated culture antigen, preserved with 1:10,000 merthiolate, is injected intradermally into patients it gives just as marked reactions as does the Frei antigen. When the twenty-third subculture in one series was heated for antigen it was found to be just as active as antigen prepared from earlier subcultures. As in the case with Frei's antigen, the culture antigen gives no reaction in normal individuals.

An attempt has been made to produce lymphogranuloma inguinale antiserum from a goat with heated culture antigen. Beginning on Dec. 1, 1933, and continuing to March 28, 1934, a total of 81 c.c. of the antigen was injected into the animal subcutaneously. The animal was bled on April 6, and 145 c.c. of serum was obtained. Merthiolate, in 1:10,000 concentration was added as preservative. Gottlieb[4] reported experimental evidence to show that there are in the serums of patients recovering from lymphogranuloma inguinale substances which have neutralizing properties on the Frei antigen when this is used for skin tests. In our work intradermal injections were made into lymphogranuloma inguinale patients of (1) Frei antigen, (2) saline, (3) Frei antigen plus normal goat serum, (4) Frei antigen plus antilymphogranuloma inguinale goat serum, after the mixture had been kept under refrigeration for forty-eight hours. (1) and (3) gave positive reactions while (2) and (4) were negative. This antiserum from the goat has been used for the treatment of two patients, reported below.

Through the courtesy of the Department of Dermatology of the Cincinnati General Hospital we are able to present thirteen cases we have treated using heated culture antigen as a vaccine administered subcutaneously, and two cases treated with antiserum. Eleven cases, including one serum-treated case, showed such marked improvement that they were considered cured in an average of eight weeks. Usually the vaccine is given with an initial dose of 0.2 to 0.3 c.c., which gives strong local reaction. Each subsequent injection produces a smaller and smaller response. The tolerance to larger doses given later is believed to be due to desensitization. Accompanying the clinical improvement there was a marked reduction in skin sensitivity. Of the remaining four cases, three are chronic and one has been treated for only two weeks. These appear to be following a similar favorable course. Beneficial therapeutic effects following the intradermal inoculation of Frei's antigen have been reported recently by Wien and Perlstein[5] who refer to the previous work of Herman and Gay-Prieto. Brief accounts of several cases follow. Treatment of four cases with heated culture antigen.

CASE 1.—E. T., negro male, single, twenty years of age. He was first seen on Dec. 14, 1933. There were two chains of swollen glands in the right groin, measuring 5 by 2 cm., and 7 by 2.5 cm. There were no palpable masses in the left groin. Patient first noticed the "swelling of groin" in latter part of September, 1933, and had been under treatment since October 4 by various physicians. There was no history of syphilis and Wassermann reaction was negative. Gonococcus infection one year ago. Frei skin tests strongly positive. Beginning December 18, with initial dose of 0.1 c.c., a total of 22.7 c.c. of vaccine was given, in 30 inoculations. Considerable reduction in skin sensitivity was obtained. On March 8, when the last inoculation was given, both chains of nodal masses had disappeared completely. No remission has occurred in two months.

CASE 2.—T. D., white male, single, twenty-seven years of age. He was exposed to infection Dec. 17, 1933. Onset of swelling in left groin was first noticed Jan. 2, 1934. Admitted to hospital January 19, and seen on January 31. There was tenderness on palpation of the affected glands, which measured 6 by 3 cm. The skin was attached to the

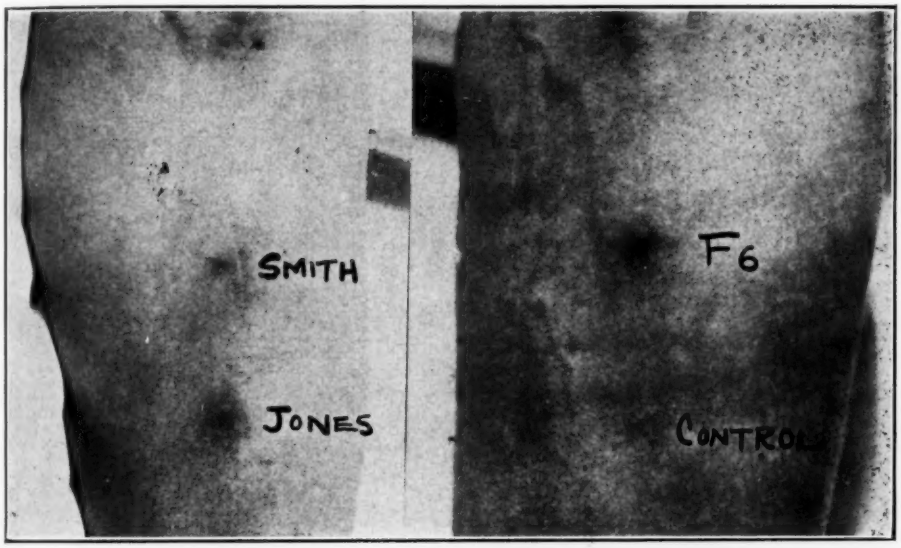


Fig. 3.—Frei skin tests, Case T.D., *Smith*, Antigen prepared from Smith. *Jones*, Antigen prepared from Jones. F_6, Culture antigen (sixth subculture). *Control*, Tissue Tyrode medium.

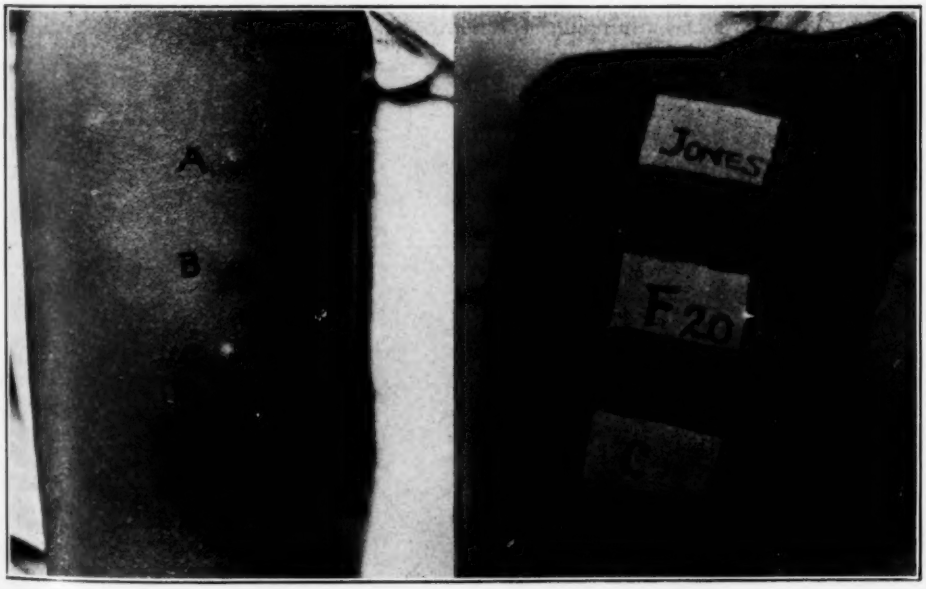


Fig. 4.—Left, Case R.S. *A*, Frei antigen. *B*, Frei antigen. *C*, Culture antigen (sixth subculture). *D*, Control with tissue Tyrode medium. Right, Case E.T. *Jones*, Frei antigen. F_{20}, Culture antigen (twentieth subculture). *C*, Control, tissue Tyrode medium.

underlying nodes, dark reddish purple in color. No masses in right groin. No history of syphilis, and Kahn test negative. Frei skin tests markedly positive. The nodes suppurated and pus was aspirated on February 14, 3 c.c., and again 2 c.c. on February 20. Bacteriologic examinations of the pus, made twice, were entirely negative. Beginning Feb-

ruary 1 and continuing to April 5, 25 subcutaneous injections of vaccine were given, totaling 22 c.c. Skin sensitivity was reduced to a negligible degree. When last seen, on April 18, there were no palpable masses.

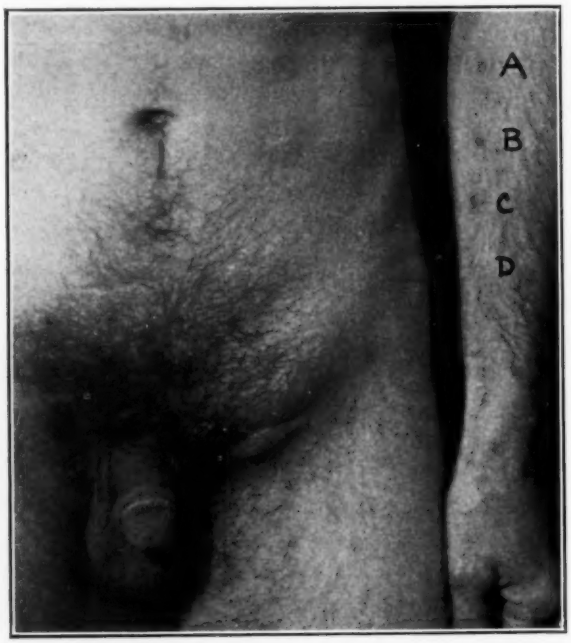


Fig. 5.—Unilateral lymphogranuloma inguinale. Positive Frei reactions on arm. *A*, Frei antigen. *B*, Culture antigen (fifteenth subculture). *C*, Frei antigen. *D*, Control with tissue Tyrode medium.

Fig. 6.—Unilateral lymphogranuloma inguinale; Case R.S., aged seventeen. Arrow points to characteristic primary lesion.

TABLE I*

GIVING DATA ON TEN CASES NOT REFERRED TO IN TEXT

CASES	AGE, SEX, COLOR	DATE OF ONSET	INCUBATION PERIOD (DAY)	DURATION (WEEK)	PRIMARY LESION	INGUINAL ADENITIS	SUPPURATION	FISTULATION	LENGTH OF TREATMENT (WEEK)	NUMBER OF INJECTIONS	TOTAL VACCINE GIVEN (C.C.)	WASSERMANN OR KAHN REACTIONS	RESULTS
R.S.	17 M. B.	12/25/33	10	4	+	U	+	+	10	27	28.4	–	C
A. R.	19 M. B.	1/19/34	14	3	+	U	+	+	9	26	27.0	–	C
H. K.	19 M. B.	2/ 8/34	7	8	–	B	–	–	5	11	8.3	–	C
C. F.	28 M. W.	3/15/34	10	2	–	U	+	+	7	21	16.0	–	C
R. M.	31 M. B.	2/19/34	9	6	–	B	–	–	6	17	10.0	–	C
S. B.	35 M. B.	3/ 9/34	?	3	+	U	+	L	8	16	11.5	–	C
B. R.	18 M. B.	July, '33	?	24	?	B	+	+	17	40	32.0	–	G. I.
H. S.	46 M. B.	Jan., '34	?	10	?	U	+	+	8	21	16.0	?	I
G. S.	26 M. B.	4/22/34	14	2	+	U	+	L	2	3	Anti-Serum 60.0	?	G. I.
J. W.	39 F. B.	July, '33	?	36	?	U	+	+	9	27	16.3	–	G. I.

*Keys to the Table: U, Unilateral. B, Bilateral. C, Cured. I, Improved. L, Lanced. I and G. I., still under treatment. G. I., Greatly Improved.

CASE 3.—C. S. J., negro male, single, thirty-one years of age. He had had inguinal adenitis for about two weeks when he was first seen, on December 28. Markedly swollen, fluctuant nodes in the right groin. No masses in the left groin. He had had gonorrhea four years ago and again four months ago. No lesion on or discharge from the penis. Kahn reaction 2-plus. Frei tests markedly positive. He was admitted to the hospital December 28. On the next day 5 c.c. of pus were aspirated from the fluctuant nodes. Dark-field examination for treponemas was negative; cultures negative. The suppurating nodes ruptured spontaneously January 2. Patient discharged from hospital January 12 and readmitted January 29. The nodes were very swollen and tender and there was a small fistula from which purulent material was discharging. Vaccine therapy was begun January 29, with initial dose of 0.3 c.c. A total of 25.5 c.c. of vaccine was injected in 28 inoculations. Patient resumed his occupation as a porter on March 17, and when last seen on April 9 the fistula was healed and masses of nodes were no longer palpable.

CASE 4.—F. M., negro male, married, thirty years of age. He was seen January 11. He had bilateral inguinal swellings. Indurated masses of nodes measured 6 by 2 cm. on left, and 7 by 2.5 cm. on right. The swelling was first noticed by the patient on December 25.

Ten days preceding the onset he was exposed to infection. There was no sore on the penis. Gonorrheal infection two years previously. Kahn reaction negative. Frei skin tests strongly positive. The nodes never suppurated. Beginning January 13, a total of 19 c.c. of vaccine was given in 22 subcutaneous injections. Marked desensitization was obtained. The masses of nodes had completely disappeared from both groins by March 5. He resumed his occupation as a porter on this date, and has had no remission for three months.

Treatment of one case with goat antiserum.

CASE 5.—C. R., white male, single, 25 years of age. He was seen first February 8, with markedly swollen nodes in left groin, measuring 10 by 5 cm., very tender and painful. Swelling of groin was first noted January 26, a week or ten days after he was exposed to infection. No primary lesion found. Slight urethral discharge, which upon examination was found to be gonorrheal. Kahn reaction 3-plus. Frei skin tests strongly positive. The involved nodes suppurated and pus aspirated: February 17, 3 c.c.; February 19, 3 c.c.; February 24, 6 c.c.; February 26, 6 c.c., and March 1, 8 c.c. Bacteriologic examination of the pus negative. Patient was admitted to hospital March 1. Beginning February 10, 9.4 c.c. of vaccine was given subcutaneously in 13 inoculations. On March 8, the nodes were incised and curetted, when vaccine therapy was discontinued. Seropurulent discharge continued and wound did not heal. Swelling of suprapubic nodes noted April 7. Patient was dismissed from hospital on April 10, readmitted on April 18, when the suprapubic nodes were incised and curetted. Continuous drainage followed. Skin test with a goat serum was negative on April 23; followed by 20 c.c. of antiserum intravenously. Twenty cubic centimeters of antiserum was given again on the twenty-fourth, and 15 c.c. by vein on the twenty-sixth. Serum sickness occurred on the twenty-ninth and lasted thirty-six hours. Patient was discharged from hospital May 4. Drainage had ceased and epithelialization had occurred. Two weeks after the first serum injection the wound was completely healed.

SUMMARY

When pus from the lymphogranuloma inguinale is planted in the medium devised by Maitland and his coworkers for the cultivation of Vaccinia virus, the medium becomes cloudy. The etiologic agent produces a cloudiness which is transmissible in serial cultures, or serial cultures alternating with guinea pig inoculations. The etiologic agent in pus or in cloudy supernatant culture fluid passes the Berkefeld "N" filter. The virus is stainable by Hosokawa's eosin-Giemsa method. The heated cultures have been used successfully in making diagnosis by the intradermal skin test, and in inducing recoveries by subcutaneous inoculations. Also heated cultures have used to produce an antiserum. Although one can draw no definite conclusions from the few cases treated with antiserum, the results in these cases justify further trial of serum therapy.

REFERENCES

1. Tamura, J. T.: Preliminary Note on the Cultivation of the Virus of Lymphogranuloma Inguinale; Its Use as a Vaccine and in the Production of an Anti-serum, J. A. M. A. **103**: 408, 1934.
2. Maitland, H. B., Laing, A. W., and Lyth, R.: Observations on Growth Requirements of Vaccinia Virus in Vitro, Brit. J. Exper. Path. **13**: 90, 1932.
3. Taniguchi, T., Hosokawa, M., Kuga, S., and Fujino, T.: A New Method of Staining Viruses of Variolo-Vaccinia and Varicella, and the Nature of Cell-Inclusions in Virus Diseases, Jap. J. Exper. Med. **12**: 91, 1934.

4. Gottlieb, F.: Neutralisation du virus de lymphogranuloma inguinale par le sérum des malades en voie de guérison, Compt. rend Soc. de biol. **111**: 141, 1932.
5. Wien, M. S., and Perlstein, M. O.: Intradermal Treatment of Lymphogranuloma Inguinale, Arch. Dermat. & Syph. **28**: 42, 1933.

WATER STUDIES IN OBESITY*

ELMER C. BARTELS, M.D., SPRINGFIELD, ILL., AND BENJAMIN BLUM, M.D., ROCHESTER, MINN.

REFERENCES have been made in the literature to disturbed water metabolism in obesity. Bauer mentioned the frequent association of obesity and disturbance of the water and salt balance. Grafe noted the tendency of obese patients toward water retention. Clasen suggested that this disturbance of water balance was due to the influence of the glands of internal secretion.

The question of water balance arises whenever reduction of weight of obese patients is attempted. Newburgh and Johnston, and Newburgh and Lashmet have reported the tendency of obese patients who are on a low caloric diet to retain water which prevented loss of weight and in some cases caused a gain of weight for some days. However, in the long run the body weight almost always approached the predicted figure as an accurate tabulation of the water exchange indicates that body weight is the result of two factors, namely: (1) gain or loss of tissue, and (2) gain or loss of water. Grote and Calo advised the study of water metabolism as a guide in the selection of therapeutic methods of reduction. Evans and Strang felt that this water retention during attempts at reduction is due to the spontaneous alteration in the capacity of fat to store water. So prominent is this matter of water retention that diuretics have been considered and used by Grafe and Zolatareva and Kogan as an adjunct to a low calorie diet. In twenty-eight of thirty-one cases Zolatareva and Kogan, by the use of 1 to 2 c.c. of mersalyl, obtained a considerable increase in the amount of urine. This sometimes reached 3 liters a day. None of these patients was suffering from cardiac disturbances. Grafe restricted the fluid intake and counteracted this tendency to water retention by the use of merbaphen.

TESTS OF DILUTION AND CONCENTRATION

As an approach to this fundamental problem we reviewed the work on dilution and concentration tests in diseases other than those of the kidney. Pratt was the first to study this subject in this country. From studies on control subjects, he concluded that in normal individuals the output of urine in four hours should be equal to or greater than the intake at the onset. No series of obese cases was considered. Buck and Proger conducted similar experiments.

*Read before the Annual Meeting of the Staff of St. Luke's Hospital, Duluth, Minnesota, January 13, 1934.
Received for publication, June 9, 1934.

The average output of urine in four hours after the ingestion of 1 liter of water was 1,122 c.c. In fifteen cases the output exceeded 1,500 c.c. in four hours. Of these, there were twelve cases of hypertension, one of neurasthenia, one of cardiosclerosis, and one of secondary anemia.

Janney and Walker, testing water balance in pregnancy, found that normal control subjects who had ingested 1,200 c.c. of water eliminated 1,353 c.c. in four hours, whereas during pregnancy the output declined progressively among clinically normal, pregnant women in the last twelve weeks of pregnancy. He attributed this to a lower functional capacity of the kidney in the latter weeks of pregnancy and considered this as a precipitating factor in toxemic states.

Malmud was the first to make studies on the disturbance in the dilution tests of Volhard in obesity. She carried out the test in various types of obesity, giving 1,000 c.c. of warm tea flavored with sugar and collecting the urine for the next four hours. She found disturbances in the output of urine which were in proportion to the severity of the obesity. The disturbance in water balance was manifested by reduction in the output of urine during four hours. A critical study of her report leaves one in doubt as to the validity of her conclusions.

Our study was precipitated by the following case which was studied in the hospital during the management of weight reduction.

REPORT OF CASE

The patient was a female, forty-four years of age, who was 65 inches tall and weighed 265 pounds. The present weight had been maintained for several years. She had had a severe type of diabetes for many years which was completely controlled by diet and 90 units of insulin a day. We felt that if the weight could be reduced a corresponding improvement in the diabetes would take place.

Physical examination, except for the marked obesity, was negative. There was no edema of the extremities. The blood pressure was and had always been within normal limits. The laboratory tests which included urine, blood counts, and blood chemistry gave normal results. Before beginning a dietary regimen a dilution test was done, which consisted in giving the patient 1,500 c.c. of water at 8:00 A.M. after a relatively dry breakfast. The urine was collected hourly for the next four hours. The following results were obtained: At the end of the first hour 170 c.c. of urine with a specific gravity of 1.009 had been excreted; during the second hour 280 c.c. with a specific gravity of 1.006; during the third hour 140 c.c. with a specific gravity of 1.007, and during the fourth hour 115 c.c. with a specific gravity of 1.008. A total of 705 c.c. of urine was excreted in the four hours.

This total was markedly below the reported normal in which intake equals output. As the urinalysis and tests of blood chemistry had given normal results, and there was apparently no edema or dehydration, we felt that we were justified in explaining this result as a disturbed water balance. Some criticism may be made on the fact that we performed only one such test before starting the patient on the reduction diet. The part the associated diabetes played in the output of urine can only be suggested.

The patient, while being up and around the entire day, was placed on a diet of 709 calories. Daily studies were made of weight, water intake and output. On the second day of the diet the patient had a marked spontaneous diuresis with a corresponding loss of weight of 2½ pounds (Table I). This sudden loss of weight can be attributed to the sudden stopping of the large dose of insulin, as described by MacBryde. For on the day the diet was

TABLE I

STUDIES OF DAILY WEIGHT, WATER INTAKE AND OUTPUT OF A PATIENT ON A DIET OF 709 CALORIES

DATE	DAYS ON DIET	DAILY CHANGE IN WEIGHT POUNDS	FLUID INTAKE C.C.	FLUID OUTPUT C.C.
July 17	1	265	600	875
July 18	2	265 to 262½	1,200	2,500
July 19	3	262½ to 260	1,275	850
July 20	4	260 to 259	1,830	1,500
July 21	5	259 to 258	1,200	1,350
July 22	6	258 to 257	1,175	775
July 23*	7	257 to 253	1,275	2,450
July 24	8	253	1,200	650
July 25	9	253 to 254½	1,000	1,000
July 26	10	254½	1,200	600
July 27	11	254½ to 253½	1,200	1,400
July 28*	12	253½ to 249½	1,200	3,200
July 29	13	249½ to 250	1,510	1,300
July 30	14	250 to 249	1,160	950
July 31	15	249		
August 1*	16	249 to 245	1,200	4,000
August 2	17	245	1,165	1,600
August 3	18	245	2,700	3,100
August 4	19	245		
Total			22,090	28,100†

*2 c.c. of salyrgan intravenously.

†Difference between total intake and output of fluid is equal to 13 pounds.

started, because of the low carbohydrate intake of approximately 60 gm. we were able to discontinue the insulin completely without subsequent sugar in the urine and with a normal blood sugar. On the seventh day edema of the ankles was first noticed. As such edema seemed to need diuretic therapy, the patient was given 2 c.c. of salyrgan intravenously. There was a subsequent diuresis of 2,450 c.c. on an intake of 1,275 c.c. and a loss of weight of 4 pounds. The edema was apparently relieved. The edema recurred on the twelfth day so 2 c.c. of salyrgan was given again. This was followed by diuresis of 3,200 c.c. on an intake of water 1,200 c.c. Weight decreased 4 pounds. After another four days this procedure was repeated with an output of urine of 4,000 c.c. on an intake of 1,200 c.c. of water. An additional loss of weight of 4 pounds occurred. The output of 1,600 of urine on the seventeenth day after the diet was begun was thought to be latent response to the salyrgan. The output of 3,100 c.c. of urine on the eighteenth day in spite of intake of 2,700 c.c. of water could not be explained at that time; however, we were able to make a plausible explanation later.

The total intake of fluid for the nineteen days in hospital was 22,090 c.c. and the total output was 28,100 c.c. The output exceeded the intake by 6,010 c.c. (13 pounds). If this is added to the actual weight (245 pounds) a total of 258 pounds is obtained which is approximately 2 pounds more than the calculated weight of 256 pounds. This indicates that the forced weight loss was entirely at the expense of the body fluids and explains the reason for the ultimate outcome in which the actual loss and the calculated loss of weight are almost identical at the end of ten weeks (Table I).

In one month, after the patient had lost consistently a total of 30 pounds, another dilution test was carried out with the following results: The output of urine for the first hour was 535 c.c. with a specific gravity of 1.005; for the second hour 1,135 c.c. with a specific gravity of 1.004; for the third hour, 160 c.c. with specific gravity of 1.006; and for the fourth hour 80 c.c. with a specific gravity of 1.009. The total output for the four hours was 1,910 c.c.

This excessive response, we felt, was due entirely to the diuretic action of water. The low caloric diet had caused a disturbance in the water balance and the large intake of water

was sufficient to start diuresis. This suggested to us the apparent benefit of taking large quantities of water during obesity to force loss of weight for psychic reasons instead of a reduction in fluid intake as advised by some authors. However, this rapid forced loss of weight by the use of diuretics did not materially affect the ultimate loss as is shown by Fig. 1. This patient has continued on the diet and the loss of weight has been constant and follows closely the estimated loss.

METHOD

We were forced from this case to accept the presence of a disturbed water balance in obese patients during efforts at reduction by the use of a low caloric diet. Our problem was to determine the water balance in obese persons not on a reduction diet and to see if it was at all disturbed as has been suggested. To do this we made determinations on the members of the nursing staff of two large Duluth hospitals and added to this a group of obese persons which included private and dispensary patients. From this number we were able to obtain a large list of normal control subjects as well as subjects who were

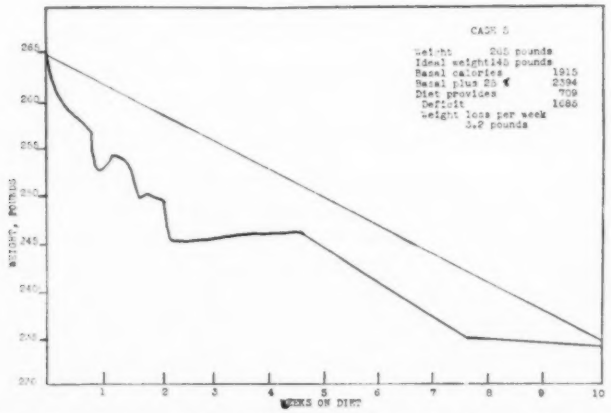


Fig. 1.—Upper line represents the estimated weight; the lower line is the actual weight. The black dot at 258 pounds at about three weeks on diet is the actual weight plus the weight of the excess water which had been lost.

under- and overweight. The entire group consisted of 102 subjects on whom 117 determinations were made. The test consisted of giving 1,500 c.c. of water at 8:00 A.M. and collecting the urine at hourly intervals for the next four hours during which time the subjects were up and around carrying on their usual duties. The amounts collected each hour, their specific gravity, and the total output were ascertained in each case. A routine urinalysis was made on the concentrated fourth hour specimen. Only those cases were included in the final consideration in which urinalysis and blood pressure were normal and physical examination was grossly negative. The subjects were normal except for the variations in weight. All were females, but we felt that this should not invalidate the results.

The age, weight, and height of each subject were taken and the normal weight was ascertained from an accepted table of normal weights. From the actual weight and the normal weight the percentage over- and underweight was calculated. We took as normal all those subjects who were within the range of 10 per cent overweight and 10 per cent underweight. Those subjects who

were more than 10 per cent overweight and those more than 10 per cent underweight were compared with the normal controls. The percentage of excretions of more than 1,500 c.c. and less than 1,000 c.c. was determined for the different groups.

RESULTS

From the tabulations in Tables II, III, and IV, it is at once seen that there is a wide variation in the hourly and total outputs in the four groups. In the obese group with the three and four determinations for Subjects 109, 110, 111,

TABLE II

RESULTS OF DILUTION TESTS ON SUBJECTS WHO WERE UNDERWEIGHT

UNDERWEIGHT POUNDS	CASE NUMBER	FIRST HOUR OUTPUT C.C.	SECOND HOUR OUTPUT C.C.	THIRD HOUR OUTPUT C.C.	FOURTH HOUR OUTPUT C.C.	TOTAL OUTPUT C.C.
Seven Subjects More Than 10 Per Cent Underweight						
19.0	7	500	710	335	90	935
17.0	46	490	445	230	35	1,200
14.0	5	380	700	52	35	1,167
13.0	65	300	430	430	84	1,244
12.0	40	135	200	540	75	950
10.0	18	435	555	230	60	1,280
10.0	4	225	450	100	20	795
Twenty-Two Subjects 0 to 10 Per Cent Underweight						
12.0	74	168	540	104	40	852
12.0	6	136	224	212	35	607
9.0	49	235	730	455	40	1,460
8.0	104	800	550	290	20	1,660
8.0	21	320	615	330	55	1,320
7.0	51	300	745	200	30	1,275
7.0	37	590	550	425	100	1,665
6.0	78	310	170	120	105	705
5.0	53	480	770	460	35	1,745
5.0	42	60	460	430	135	1,085
4.0	69	330	450	520	122	1,422
4.0	61	185	380	370	70	1,005
4.0	10	240	485	480	355	1,560
3.5	101	925	250	90	250	1,515
3.5	86	200	475	550	125	1,350
3.0	95	500	475	475	100	1,550
2.5	68	380	840	370	26	1,616
2.0	76	90	400	425	85	1,000
1.0	19	400	540	75	20	1,035
1.0	8	185	610	100	30	925
0.5	29	455	245	125	32	857
0.5		740	550	132	32	1,454

112, and 113, this range is also seen. As this variation is present in all the groups we felt that if any comparison or difference was noted, it would only be secured by averaging the groups.

Table V is a complete tabulation of the average hourly output and average total output in each of the groups. We concluded that a normal output, if there is such an entity, could best be obtained by averaging the output of the groups from 0 to 10 per cent overweight and from 0 to 10 per cent underweight. The average total output of urine is then calculated to be 1,334 c.c. in four hours

TABLE III

RESULTS OF DILUTION TESTS ON FORTY-TWO SUBJECTS WHO WERE FROM 0 TO 10 PER CENT OVERWEIGHT

OVERWEIGHT POUNDS	CASE NUMBER	FIRST HOUR OUTPUT C.C.	SECOND HOUR OUTPUT C.C.	THIRD HOUR OUTPUT C.C.	FOURTH HOUR OUTPUT C.C.	TOTAL OUTPUT C.C.
0.0	52	210	680	370	327	1,587
0.0	55	630	455	410	55	1,550
0.0	89	650	700	300	90	1,740
1.5	93	200	850	350	75	1,375
2.0	25	195	680	460	45	1,380
2.0	56	410	550	240	30	1,230
2.0	59	340	590	110	35	1,075
2.0	90	425	725	650	120	1,920
2.0	92	500	700	200	100	1,500
2.5	1	260	425	300	120	1,105
2.5	85	250	850	425	75	1,600
3.0	36	200	750	250	35	1,235
3.0	63	210	420	185	30	845
3.0	84	625	850	200	75	1,750
3.0	88	500	750	400	120	1,775
3.0	102	650	600	150	50	1,450
4.0	28	225	665	430	325	1,645
4.0	66	370	480	240	40	1,130
4.0	30	435	455	145	55	1,090
4.0	47	740	455	435	35	1,665
4.0	99	300	750	200	75	1,325
4.5	2	250	750	210	30	1,240
5.0	26	870	640	180	35	1,725
5.0	43	260	565	350	210	1,385
6.0	54	870	475	135	75	1,555
7.0	23	360	400	430	235	1,425
7.0	38	490	425	330	300	1,545
7.0	45	330	620	245	60	1,255
8.0	39	125	550	600	45	1,320
8.0	57	240	570	415	170	1,395
8.0	72	460	660	550	70	1,740
8.0	94	600	600	250	50	1,500
8.5	75	530	455	96		1,081
8.5	106	600	825	250	100	1,775
9.0	7	130	544	210	24	908
9.0	50	205	225	55	35	520
9.0	67	600	580	470	105	1,755
9.0	98	600	500	510	100	1,710
10.0	27	410	395	297	40	1,142
10.0	62	290	450	370	90	1,200
10.0	91	500	800	450	180	1,930
13.0	31	385	460	560	85	1,490

after an intake of 1,500 c.c. of water. The average hourly output is 389 c.c., 545 c.c., 311 c.c., and 88 c.c. in the four hours, respectively. At once it is seen that both the group of subjects more than 10 per cent underweight and that more than 10 per cent overweight have total outputs below the normal of 1,334 c.c.; the former more than the latter. An apparently significant figure is the fourth hour output in the group more than 10 per cent overweight. This figure of 119 c.c. suggests the tendency of obese patients to have a disturbance which is not marked in total output but in the manner of output. A sustained output was always noticed by the relatively increased fourth hour output. An error, which may be suggested in this comparison of the group of overweight subjects

TABLE IV

RESULTS OF DILUTION TESTS ON THIRTY-TWO SUBJECTS WHO WERE MORE THAN 10 PER CENT OVERWEIGHT

OVERWEIGHT POUNDS	CASE NUMBER	FIRST HOUR OUTPUT C.C.	SECOND HOUR OUTPUT C.C.	THIRD HOUR OUTPUT C.C.	FOURTH HOUR OUTPUT C.C.	TOTAL OUTPUT C.C.
10	9	275	120	70	40	505
10	35	155	165	135	75	530
10	73	485	205	130	80	900
10	100	500	650	50	100	1,300
11	22	305	550	375	45	1,275
11	24	200	530	90	30	850
12	64	630	620	144	50	1,440
12	70	810	700	450	50	2,000
12	108	1,000	850	150	25	2,025
13	44	420	480	180	55	1,135
13	82	800	825	150	20	1,795
14	103	500	600	300	50	1,450
15	20	315	395	125	30	865
15	105	900	700	150	100	1,850
16	79	285	465	260	95	1,165
16	87	600	600	500	120	1,820
17	96	500	500	500	50	1,550
18	83	900	650	300	150	2,000
20	48	555	670	255	40	1,520
20	111	95	320	235	45	695
		35	200	475	350	1,060
		55	500	575	285	1,415
		195	600	350	70	1,215
21	107	550	650	400	110	1,710
22	3	350	875	210	52	1,487
22	11	165	500	145	35	845
22	60	155	375	70	50	650
24	14	550	360	175	60	1,145
29	34	250	435	490	120	1,285
29	58	350	570	290	110	1,320
31	113	395	380	455	320	1,550
		180	375	450	560	1,565
		270	470	470	235	1,445
		265	265	450	230	1,310
38	109	210	580	460	35	1,285
		100	590	435	175	1,300
		170	725	430	75	1,400
		210	605	480	220	1,515
40	110	235	620	185	40	1,080
		175	580	355	90	1,200
		250	655	240	55	1,200
46	112	240	310	330	220	1,200
		365	410	300	200	1,275
		225	395	345	205	1,170
		270	230	250	210	1,070
75	15	170	280	140	115	705

with that of underweight subjects, is that a sufficiently wide range in the amount underweight was not available for absolute comparison with the group of overweight subjects.

Table VI is an additional determination of the percentage of subjects in the four groups which had outputs of urine of more than 1,500 c.c. or of less than 1,000 c.c. Of apparent significance is the fact that only 28 per cent of the group that was more than 10 per cent overweight had an output of over 1,500 c.c. However, the difference is not great. The 7 per cent of the group

TABLE V

AVERAGE TOTAL OUTPUT OF URINE FROM EACH OF THE FOUR GROUPS

WEIGHT	HOUR				
	FIRST	SECOND	THIRD	FOURTH	TOTAL, C.C.
0 to 10 per cent underweight	364	502	304	83	1,255
0 to 10 per cent overweight	415	589	319	94	1,414
Average	389	545	311	88	1,334
More than 10 per cent underweight	338	498	272	57	1,179
More than 10 per cent overweight	359	490	296	119	1,269

TABLE VI

PER CENT OF SUBJECTS IN EACH GROUP WITH OUTPUT OF MORE THAN 1,500 C.C. AND LESS THAN 1,000 C.C. OF URINE

MORE THAN 1,500 C.C.	PER CENT
0 to 10 per cent underweight	33
0 to 10 per cent overweight	40
More than 10 per cent underweight	28
More than 10 per cent overweight	34
LESS THAN 1,000 C.C.	
0 to 10 per cent underweight	23
0 to 10 per cent overweight	7
More than 10 per cent underweight	28
More than 10 per cent overweight	28

which was from 1 to 10 per cent overweight who had an output of less than 1,000 c.c. of urine is significant in that it indicates that obese subjects retain water to a much less degree than those in the other group.

SUMMARY AND CONCLUSIONS

Disturbance in water balance does occur during attempts at reduction in weight in cases of obesity as is shown in the literature and the case reported. In obese subjects who are not on diets, there is some slight evidence of retention of water as is indicated by the Volhard dilution test. This retention of water is more marked in underweight subjects. In obese subjects there is evidence of a sustained output, as indicated by the moderately high output of 119 c.c. as against a normal output of 88 c.c. in the fourth hour, as well as a slight reduction in total output. Obese subjects do not retain fluids to a great degree, as in only 7 per cent was the output less than 1,000 c.c. Underweight subjects have smaller total outputs in four hours than obese subjects.

REFERENCES

1. Bauer, J.: Endocrine Obesity, J. A. M. A. **93**: 2042, 1929.
2. Buck, R. W., and Proger, S. H.: The Dilution and Concentration Test of Renal Function: Comparison of Results With Other Test in 100 Cases, New Eng. J. M. **203**: 1283, 1930.
3. Clasen, A. C.: Obesity, J. A. M. A. **95**: 154, 1930.
4. Evans, F. A., and Strang, J. M.: The Treatment of Obesity With Low Caloric Diets, J. A. M. A. **97**: 1063, 1931.
5. Grafe, E.: Theorie und Praxis der Entfettungskuren, Med. Klin. **25**: 373, 1929.
6. Grote, F., and Caló, G.: Über Entfettungskuren, Schweiz. med. Wchnschr. **60**: 579, 1930.

7. Janney, J. C., and Walker, Elisabeth W.: Kidney Function in Pregnancy. I. Water Diuresis in Normal Pregnancy, J. A. M. A. **99**: 2078, 1932.
8. MacBryde, C. M.: Insulin Resistance in Diabetes Mellitus, Arch. Int. Med. **52**: 932, 1933.
9. Malamud, Teresa: La prueva del agua o de diluçion de Volhard en las obesas, La Prensa méd. (Argentina) **20**: 1142, 1933.
10. Newburgh, L. H., and Lashmet, F. H.: The Importance of Dealing Quantitatively With Water in the Study of Disease, Am. J. M. Sc. **186**: 461, 1933.
11. Newburgh, L. H., and Johnston, M. W.: Endogenous Obesity: a Misconception, J. Am. Diet. Assn. **5**: 275, 1930.
12. Pratt, J. H.: The Dilution and Concentration Tests of Renal Function, Boston M. & S. J. **195**: 203, 1926.
13. Zolatareva, H., and Kogan, T.: Mersalyl Treatment of Obesity, Russhaya Klin. (Moscow) **13**: 606, 1930.

LABORATORY METHODS

A MODIFIED METHOD FOR THE DETERMINATION OF BLOOD CHOLESTEROL*

JEROME E. ANDES, PH.D., NEW ORLEANS, LA.

IN THE determination of the cholesterol content of the blood and other body fluids, two general procedures are available. The first group of procedures comprises those in which the cholesterol is precipitated with digitonin, and estimated by either colorimetric or gravimetric means. In the second group the blood or serum is dried on a suitable medium, the cholesterol (and other fatty substances) extracted with a suitable fat solvent, and the cholesterol estimated colorimetrically by the Liebermann-Burchard reaction. The digitonin method, either as described by Windaus,[1] or according to one of the legion of modifications published in recent years, is probably the most accurate and reliable. However, it is quite an expensive method, and each determination requires considerable time for its completion. In addition, a fair degree of technical skill is needed for such analyses, and the average clinical technician does not possess a great amount of chemical laboratory training.

For this and other reasons the colorimetric method has become quite generally employed in clinical laboratories. The method is inexpensive, it requires a minimum amount of technical skill, and it requires much less time for each determination than does any of the digitonin methods. Also the values obtained are sufficiently accurate for clinical work.

Although Bloor[2] described the first direct colorimetric method, applicable to clinical work, the method of Myers and Wardell[3] published two years later was the first method that seemed to give satisfactory results in the hand of technicians. In this method the blood or serum is dried on plaster of Paris (at 100° C. for an hour), the cholesterol extracted with chloroform and the cholesterol in the chloroform extract determined colorimetrically by means of the Liebermann-Burchard reaction. This method, or as modified by Oser and Karr,[4] has proved to be very reliable, and is in use in many clinical laboratories at the present. The chief objections that may be offered to this method are the following: it requires apparatus not always present in clinical laboratories; at least 30 c.c. of chloroform are required for each determination; and the chloroform extract must be transferred to another vessel before making the colorimetric estimation. Lieboff[5] sought to simplify the method by the use of a special tube in which both the extraction and colorimetric estimation are made. Also he

*From the Department of Biochemistry, Louisiana State University Medical Center.
Received for publication, April 9, 1934.

used filter paper instead of plaster of Paris as a medium upon which to dry the blood, and reported values that favorably compared with those obtained by the method of Myers. The method was further modified by Ling.[6] However, Allardyce[7] found that the use of filter paper, cotton batting, and similar media as desiccating agents gave results far below those obtained when plaster of Paris was employed. This same finding was confirmed by Bernhard and Drekter.[8] They found that chloroform did not extract over two-thirds of the cholesterol from serum dried on filter paper, but they did find that an equal mixture of alcohol and ether gave a complete extraction of cholesterol from serum dried on this medium, and checked their values against digitonin determinations. These workers employed a simple extracting apparatus composed chiefly of a Folin-Wu tube and test tube condenser, and carried out the colorimetric estimation in the same tube. Such an apparatus had already been employed by Harnes.[9]

EXPERIMENTAL

Our study of cholesterol methods was not begun with the idea of describing a new procedure, but rather with the idea of choosing the most suitable method for a problem based largely on blood cholesterol estimation. In Table I are shown some of the more important results obtained by a comparison of different extracting media, solvents, etc. The results given were chosen as representative of each particular study. The findings can be expressed very briefly as follows:

1. Whole blood or serum when dried on filter paper and extracted with chloroform gives values much lower than those obtained when the blood or serum is dried on plaster of Paris. The discrepancy in the two values is more marked with serum than with whole blood. This confirms the findings of both Bernhard and Drekter, and of Allardyce.

2. The amount of cholesterol extracted by chloroform from blood or serum dried on plaster of Paris is practically independent of the time or temperature of drying (within reasonable limits). About five minutes' time is sufficient, and the mixture may stand at least twelve hours at room temperature. However, about thirty minutes appears to be the optimum length of time. Drying at room temperature appears to give values almost identical with those obtained by drying at 100° C. and apparently the use of the electric oven is not required.

3. The cholesterol extracted by chloroform from blood or serum dried on filter paper varies with both the time and temperature of drying. Increases in temperature decrease the amount of sterols extracted in every case, if the time of drying is kept constant. Likewise if the temperature is kept constant, the cholesterol extracted decreases with the length of time of drying. Although Lieboff reports that the blood does not require drying before extraction, the author could not obtain satisfactory results in this manner.

4. The plaster of Paris employed must be fresh (not hydrated). Definitely lower values were obtained when hydrated plaster of Paris (hydrated by standing in the open air several days) was employed, especially when the blood and plaster was dried in the oven at 100° C.

TABLE I

STUDIES ON THE DETERMINATION OF CHOLESTEROL IN WHOLE BLOOD AND SERUM

(Fig. in mg. per 100 c.c.)

COMPARISON OF PLASTER OF PARIS AND FILTER PAPER AS DESICCATING MEDIUM		
SAMPLE NO.	VALUE	MEDIUM
1. (Blood)	180	Plaster of Paris
	181	Plaster of Paris
	170	Filter paper
	166	Filter paper
2. (Blood)	198	Plaster of Paris
	196	Plaster of Paris
	136	Filter paper
	133	Filter paper
3. (Blood)	193	Plaster of Paris
	132	Filter paper
4. (Blood)	141	Plaster of Paris
	125	Filter paper
5. (Serum)	135	Plaster of Paris
	80	Filter paper
6. (Serum)	148	Plaster of Paris
	81	Filter paper

EFFECT OF TIME AND TEMPERATURE WHEN USING PLASTER OF PARIS AS A DESICCATING MEDIUM		
SAMPLE NO.	VALUE	TIME AND TEMPERATURE
7. (Serum)	213	30 min. at 25° C.
	219	12 hr. at 25° C.
8. (Serum)	110	30 min. at 25° C.
	109	12 hr. at 25° C.
9. (Serum)	200	5 min. at 25° C.
	199	30 min. at 25° C.
	201	30 min. at 25° C.
	199	12 hr. at 25° C.
	200	12 hr. at 25° C.
10. (Serum)	123	30 min. at 25° C.
	119	1 hr. at 100° C.
11. (Serum)	121	30 min. at 25° C.
	120	1 hr. at 100° C.
12. (Serum)	181	30 min. at 25° C.
	180	1 hr. at 100° C.

EFFECT OF TIME AND TEMPERATURE WHEN USING FILTER PAPER AS A DESICCATING MEDIUM		
SAMPLE NO.	VALUE	TIME AND TEMPERATURE
13. (Blood)	170	30 min. at 25° C.
	151	8 hr. at 25° C.
	122	1 hr. at 100° C.
14. (Blood)	136	30 min. at 25° C.
	115	8 hr. at 25° C.
15. (Blood)	133	30 min. at 25° C.
	110	30 min. at 40° C.

NEW VS. OLD PLASTER OF PARIS (DRIED 1 HR. AT 100°)		
SAMPLE NO.	NEW	OLD
16. (Serum)	201	108
	199	105
17. (Serum)	213	161
	219	152
18. (Blood)	213	184

Comparison of Chloroform vs. Alcohol-Ether as an Extracting Medium

SAMPLE NO.	CHLOROFORM (FROM PLASTER OF PARIS)	ALCOHOL-ETHER (FROM FILTER PAPER)
19. (Serum)	278	260
20. (Serum)	146	81
	142	76
21. (Serum)	200	148
	196	189
22. (Serum)	180	165
	177	154

SAMPLE NO.	SERUM DRIED ON PLASTER OF PARIS	
	CHLOROFORM	ALCOHOL-ETHER
23. (Serum)	161	160
	160	145
24. (Serum)	178	165
25. (Serum)	121	120
26. (Serum)	221	217

5. The alcohol-ether mixture does not completely extract the sterols from blood or serum dried on filter paper,* but when the blood is dried on plaster of Paris the amount extracted is similar (although slightly below in every case) to

*We do not believe that this is necessarily contradictory to the findings of Bernhard and Drekter. The atmosphere in New Orleans is so nearly saturated with water vapor that a condensation of moisture takes place with any method employing an extraction apparatus. At the end of the period of extraction (with Bernhard and Drekter's method) from ½ to 1 c.c. of water had collected in the extraction tube. This had mixed with the alcohol-ether mixture, rendering it inefficient as a fat solvent. On a few days, when the atmosphere was somewhat less humid than usual, we were able to get results with filter paper and alcohol-ether, that closely approached those with plaster of Paris and chloroform. However, another big objection to the use of alcohol-ether is that it must be evaporated off and chloroform added before the colorimetric estimation. This evaporation requires considerable time, unless facilitated by a current of air. With the chloroform extraction the evaporation is not necessary.

that extracted by chloroform. Also the results obtained by the use of alcohol-ether as a fat solvent were more erratic than when chloroform was employed.

Other desiccating media (as fuller's earth, powdered calcium carbonate and other inert salts, and zinc dust) were tried but found to be inferior to plaster of Paris. Also other extracting media (carbon tetrachloride, absolute alcohol, ether, etc.) were tested but again found to be similarly less desirable than chloroform.

From the above results it would seem that the most satisfactory method would be one employing plaster of Paris as a drying medium and chloroform as an extraction medium. While the method of Myers and Wardell satisfies these requirements, a micromodification of this method is described below which employs an extraction apparatus (composed chiefly of a Folin-Wu tube) in which the colorimetric comparison is also made.

METHOD FOR THE DETERMINATION OF CHOLESTEROL IN WHOLE BLOOD OR SERUM

Principle.—The blood (or serum) is mixed with plaster of Paris and allowed to dry a suitable length of time at room temperature. The cholesterol is then extracted by means of a simple refluxing apparatus and the colorimetric comparison carried out in the same tube.

Procedure.—Accurately pipette* 0.25 c.c. of whole blood or serum into a 3-inch evaporating dish. Add a little over one-half teaspoonful of plaster of Paris (fresh) and thoroughly mix† with a spatula (a round-bottomed porcelain spoon is best used for this mixing). Scrape the plaster loose from the dish with a clean dry steel spatula, and cover the mixture with a watch glass and allow it to stand at room temperature for thirty minutes or longer.‡ Then by means of a "V"-shaped trough of smooth heavy paper, transfer the plaster of Paris to an extraction thimble of such a size that it will fit inside of a Folin-Wu tube§ (see Fig. 1). Place the extraction thimble inside the Folin-Wu tube, and arrange the rest of the extraction apparatus** as shown in Fig. 1. Fill the bulb of the Folin-Wu tube about two-thirds full with pure chloroform, and place the tube on the hot plate. Add more chloroform as needed (until the thimble and contents become saturated with the chloroform) and carry on the extraction for at least thirty minutes. At the end of this time remove the condenser and extraction thimble and allow about one-half of the chloroform in the bulb to boil away.††

*This is conveniently done by using a calibrated 1 c.c. pipette (graduated to 1/100 c.c.). The delivery between two marks is most satisfactory (i.e., between the 0.7 c.c. and 0.95 c.c. marks on the pipette).

†Thorough mixing is essential for accurate values.

‡It may be heated in an oven at 100°C. for an hour, or allowed to stand overnight at room temperature if desired.

§These thimbles can be purchased on the market, or can be conveniently made from library paste and thick filter paper. The thimble is molded over a small test tube, and after drying extracted with chloroform. The thimbles can be used for an indefinite number of determinations as the plaster of Paris is easily shaken out of the dry tube after each determination.

**The apparatus is similar to the one described by Bernhard and Drekter. It chiefly consists of a Folin-Wu tube where 5 c.c. of volume comes in the constricted portion. This point is marked with a sharp file. The condenser is merely a test tube and is of such a size that its rim will just prevent its falling into the Folin-Wu tube (as shown); it is best made from a Pyrex test tube of a suitable size, cut off at the proper length.

††This removes any water that might have collected during the extraction.

Then remove the Folin-Wu tube from the hot plate, allow it to cool and add chloroform to the 5 c.c. mark. Now add 5 c.c. of the standard solution of cholesterol* (containing 0.4 mg. cholesterol) into another Folin-Wu tube and add to each 1 c.c. of pure acetic anhydride. Now place both tubes in a beaker of water (at about 25° C.), and add 0.2 c.c. (5 drops) of concentrated sulphuric

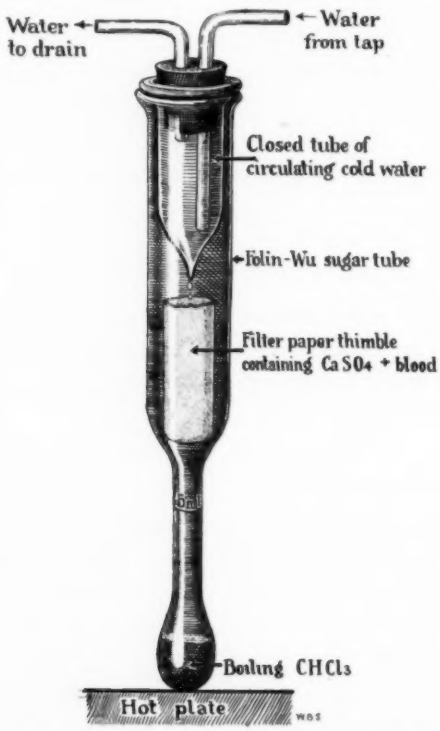


Fig. 1.—Cholesterol extraction apparatus.

acid. Mix† quickly, stopper tubes, and place the beaker and tubes in the dark. At the end of fifteen minutes compare the colors in a colorimeter.

Calculation.—The cholesterol in the blood or serum is calculated as follows:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 160 = \text{mg. cholesterol per 100 c.c.}$$

The total time for a complete determination is about ninety minutes.

SUMMARY

1. In the colorimetric estimation of blood (or serum) cholesterol, plaster of Paris seems to be the most satisfactory desiccating medium and chloroform appears to be the most suitable extracting medium.

*The stock standard is prepared by dissolving 160 mg. of pure cholesterol in 100 c.c. of chloroform. The working standard is made by pipetting 5 c.c. of this solution into a 100 c.c. volumetric flask, and making the volume up to 100 c.c. with chloroform. 5 c.c. = 0.4 mg. of cholesterol.

†The mixing is easily accomplished by blowing a current of air into the bottom of the tube. The air is dried by interposing a calcium chloride drying tube.

2. A method is described for the determination of cholesterol in blood (or other body fluids). The blood is dried on plaster of Paris, and the cholesterol is extracted with chloroform, the entire extraction and color development being completed in a Folin-Wu sugar tube.

REFERENCES

1. Windaus, A.: Über die Bestimmung des Cholesterins und der Cholesterinester in einigen normal und pathologischen Nieren, Ztschr. f. physiol. Chem. **65**: 110, 1910.
2. Bloor, W. R.: The Determination of Cholesterol in Blood, J. Biol. Chem. **24**: 227, 1916.
3. Myers, V. C., and Wardell, E. L.: The Colorimetric Determination of Cholesterol in Blood With a Note on the Estimation of Coprosterol in Feces, J. Biol. Chem. **36**: 147, 1918.
4. Oser, B. L., and Karr, W. G.: The Lipoid Partition in Blood in Health and Disease, Arch. Int. Med. **36**: 507, 1925.
5. Lieboff, S. L.: A Simplified Method for Cholesterol Determination in Blood, J. Biol. Chem. **61**: 177, 1924.
6. Ling, S. M.: The Determination of Cholesterol in a Small Amount of Blood, J. Biol. Chem. **76**: 361, 1928.
7. Allardyce, John: The Determination of Cholesterol in Blood, Canad. J. Research **3**: 115, 1930.
8. Bernhard, Adolph, and Drekter, I. J.: A Simple and Accurate Method for the Determination of Cholesterol in Blood Serum or Plasma, J. Lab. & Clin. Med. **16**: 1225, 1931.
9. Harnes, A. R.: The Colorimetric Determination of Lipoid Phosphorus in Blood, J. Biol. Chem. **77**: 405, 1928.

THE ENUMERATION OF BLOOD PLATELETS*

ISADORE OLEF, M.D., BOSTON, MASS.

THE third formed cellular elements of the blood, the blood platelets or thrombocytes, were discovered by Donné[1] in 1842 who called them "globulins." Several years later they were independently observed by Zimmermann[2, 3, 4] who applied to them the term "Elementarkörperchen" or elementary corpuscles. The classical descriptions of these bodies by Schultze,[5] Osler,[6, 7] and particularly by Bizzozero[8, 9] placed them on an important hematologic basis. Bizzozero who described these cellular elements during life as they appeared in the mesenteric vessels of rabbits and guinea pigs, introduced the term "blood platelet."

The blood platelet count and its variations occupy an important part in hematology in view of the rôle these bodies play in blood coagulation, thrombus formation and also possibly the defense of the organism against infection by microorganisms. The platelets are probably the most susceptible of any of the formed blood elements to mechanical or chemical influences, and are quickest to regenerate when favorable conditions supervene (Doan and Sabin[10]). The enumeration of the thrombocytes is, therefore, important in the study of various hemorrhagic diseases, particularly purpura hemorrhagica; it probably is of greater significance than is realized in various anemias, leucemias and infections.

Numerous methods for counting blood platelets have been introduced since the time of Bizzozero. The difficulties encountered by various investigators in counting platelets are undoubtedly due to the peculiar physicial properties of these cellular elements; viz., their great tendency to agglutination, adhesion, and easy disintegration under extravascular conditions. To these must be added their small size and light weight. Although the number of methods for enumerating the thrombocytes is large, none is fully satisfactory.

In 1930, I[11] described an indirect method for counting blood platelets, the diluting fluid consisting of a 2 per cent solution of sodium metaphosphate (Merck). Shortly after the publication of my method it was discovered that Merck & Co. had discontinued the manufacture of this chemical. A 2 per cent solution of a soluble polymer of sodium metaphosphate prepared by Howe and French of Boston was found to be too hypertonic for platelet counting. Some twenty different concentrations of this chemical, alone and in combination with other substances, were then experimented with. In addition a number of solutions that have been recommended for platelet counting were tried: physiologic saline, 14 per cent solution of magnesium sulphate, several different concentra-

*From the Medical Clinic of the Boston Dispensary, Service of Dr. Joseph H. Pratt, and the Department of Medicine, Tufts Medical School.

Received for publication, April 21, 1934.

This study was made possible by a grant from the Bingham Associates Fund.

tions of formaldehyde, sodium citrate, Kemp and Calhoun's solution,[12] Dameshek's solution,[13] and the solution of Rees and Ecker.[14] The older physiologic solutions of Locke and Ringer, and the later modifications by Hédon and Fleig,[15] Adler[16] and Tyrode[17] were also used as diluents. The last named solution, with slight modifications, was used by Flössner[18] and Hofmann[19] for platelet counting. As diluents the physiologic solutions were found to be very unsatisfactory because they preserve the platelets for only fifteen to twenty minutes and because they either contain preformed artefacts or produce them on contact with blood. A preserving fluid, to be satisfactory, must answer the following requirements: (1) It must preserve the platelets for a reasonable length of time, at least several hours; (2) it must preserve the other cellular elements of the blood, the erythrocytes and leucocytes; (3) it must be isotonic so that the formed blood elements are not distorted; (4) it must be free from particulate matter; and (5) it must not produce precipitates or colloidal bodies when mixed with blood. The sodium metaphosphate solutions, first used by Pratt[20] for preserving and enumerating platelets, in general were found to be more satisfactory than any of the other diluting fluids. That sodium metaphosphate is an excellent preservative for blood platelets as well as the other cellular blood elements has been pointed out by many investigators (Deetjen,[21, 22] Hirschfeld,[23] Sabbatani,[24] Helber,[25] Pratt,[26] Richardson,[27] Cadwalader,[28] Gottlieb,[29] Aynaud,[30] Reid,[31] Maixner and von Decastello,[32] Degkwitz,[33, 34] Schenk,[35] Wittkower,[36] and Bannerman.[37]). The following solution was empirically found to be the most satisfactory:

	GM. OR C.C.
Sodium metaphosphate (Howe and French)	1.0
Sodium chloride	0.5
Dextrose	0.1
Distilled water	100.0

In this solution the platelets appear as clear, isolated, highly refractile bodies with numerous fine spinelike processes projecting from the periphery. The erythrocytes and leucocytes are excellently preserved; crenated or distorted red cells are rarely seen. It was found entirely superfluous to employ a dye to stain the platelets. However, if it is desirable to stain them, the following formula may be used:

	GM. OR C.C.
Sodium metaphosphate	1.0
Sodium chloride	0.4
Dextrose	0.1
Sodium bicarbonate	0.1
Brilliant cresyl blue	0.15
Distilled water	100.0

In this solution both the platelets and reticulocytes are well stained so that, as has been suggested by Dameshek,[13] a reticulocyte and thrombocyte count can be done simultaneously. The solutions, if not in use, should be kept in a cold place and should be filtered every few days.

TECHNIC FOR ENUMERATING THE BLOOD PLATELETS

The technic for counting blood platelets is more difficult than that of any other cellular constituent of the blood in view of the readiness with which they

disintegrate outside the body and the way they adhere to injured vascular epithelium and to foreign substances. The important technical steps in counting platelets are: (1) Drawing of the blood, (2) diluting and preserving the blood and (3) properly enumerating the platelets in the diluted blood. The first two procedures will be considered now.

OBTAINING BLOOD FOR PLATELET COUNT

According to Schenk,[35] Zeller,[38] Pagniez and Mouzon,[39] Kristenson,[40] Horwitz[41] and Jürgens[42] capillary blood is as accurate as venous blood for platelet counting and is certainly easier to obtain. A number of investigators (Aynaud,[30] Achard and Aynaud,[43, 44] Thomsen,[45] Buckman and Hallisey,[46] Cramer and Bannerman,[47] Gutstein,[48] Preiss,[49] and Roskam[50]) recommend the use of venous blood in order to avoid admixture of tissue juices and the adhesion of platelets to raw surfaces. This, however, is easily obviated by puncturing the finger and following the procedure to be described. Moreover, the methods employing venipuncture involve too much manipulation of the blood and do not yield higher counts than the methods in which capillary blood is used.

The finger should always be used for drawing blood. It is advisable to employ a warm hand-bath immediately before puncturing the finger. This is carried out by immersing the hand in warm water, the hand being opened and closed for exercise. The active hyperemia thus produced accelerates the local circulation, facilitating a rapid flow of blood when the finger is punctured. The palmar surface of the finger tip is punctured with a Frank automatic lancet about one centimeter from the end after thorough cleansing of the parts with soap and water and subsequent drying with alcohol and ether or acetone. The lobe of the ear is not a satisfactory site for drawing blood; here the external temperature is apt to produce considerable changes in the capillaries, as has been pointed out by Naegeli.[51] Moreover, the presence of fine hair in that region favors the adhesion and agglutination of platelets. I[11] have shown that blood obtained from the finger yields higher platelet counts than blood obtained from the lobe of an ear.

PRESERVING AND DILUTING THE BLOOD

Extravascular changes in thrombocytes have been studied by a number of investigators, but just what the earliest changes are has never been determined. It is generally conceded, however, that the early changes are hastened by the admixture of tissue juices and retarded by the addition of anticoagulants and by dilution. When the blood platelets come in contact with a water-wettable surface, they become adherent to it, spread out, and rapidly disintegrate, liberating thromboplastic substances which are capable, in the presence of calcium salts, of transforming fibrinogen into fibrin with the formation of thrombin. If the water-wettable surface is smaller than that of the platelets, as in the case of colloids or microorganisms, the platelets attach themselves to it without disintegrating rapidly. The two important physicochemical processes involved in some of these changes are discussed by Morawitz and Brugsch.[52] One is based

on the theory that each platelet possesses a negative electric charge whose magnitude depends on a number of factors, as the hydrogen ion concentration of the blood or the composition of the blood plasma. An excess of substances like fibrinogen or globulin causes a diminution in the electric charge, thus favoring agglutination and disintegration of the platelets (Starlinger and Sametnik,[53] Stuber and Lang[54]). This is further favored by contact with foreign substances and chemical injury of various types (Dietrich[55]). The other is based on the theory originally proposed by Achard and Aynaud[56] and further elaborated by Roskam[57] and Govaerts[58, 59, 60, 61] that there is adsorbed on the surface of each platelet a thin layer of plasma colloids, the so-called "plasma layer," which brings about agglutination and disintegration of platelets when the latter come in contact with water-wettable surfaces or foreign bodies. Osterhout[62] points out that when animal tissues are crushed or injured so that the internal fluid of the cells escapes, the fluid thus liberated may have a deleterious action on the healthy cells.

It is obviously important to bring the blood platelets rapidly in contact with the preserving fluid in order to obviate the destructive effects incident to the admixture of tissue juices, disintegration products of injured cells and of foreign bodies. This can be accomplished by (1) thorough cleansing of the skin before the puncture, (2) discarding the first drop or two of blood, (3) placing a drop of the preserving fluid over the puncture wound before the blood reaches the surface of the skin, and (4) mixing the blood and preserving fluid in paraffin or amber vessels. The discarded blood contains disintegration products of crushed and injured cells and is also diluted by the admixture of lymph; the subsequent drops of blood contain no such disintegration products and no admixture of lymph, since the higher pressure within the blood vessels very promptly forces the blood into the open lymph channels. Moreover, Pemberton,[63] Pierce and Pemberton[64] and Crouter and Cajori[65] have shown that in a considerable percentage of normal persons the first drop of blood yields higher erythrocyte counts than the subsequent drops; the reverse is true, they claim, in patients with arthritis. Obviously, when the indirect methods for counting platelets are employed where the erythrocyte-platelet ratio is determined, the first drop of blood should be discarded in order to obtain standard and comparable results. Horwitz[41] obtained higher platelet counts in the first drop of blood than in the subsequent drops; Steinmaurer[66] obtained no regular variations in the number of thrombocytes in the first and the fourth to fifth drops, although his average counts reveal somewhat greater numbers in the fourth to fifth drops of blood than in the first drop. In my own counts the first drop of blood has always yielded lower platelet values than the subsequent drops. The puncture wound should always be made sufficiently deep to obtain a fairly rapid flow of blood without the application of pressure. Bannerman[37] has shown that blood flowing rapidly yields higher platelet counts than blood flowing slowly. After the first drop or two of blood has been wiped away the further flow of blood can be easily controlled by gentle pressure over the wound with a piece of sterile gauze until the preserving fluid is applied.

Blood thus obtained from the finger must be mixed in the proper proportion with some diluent which not only preserves the platelets, but also the other cellular constituents of the blood. The use of undiluted blood for platelet counting, recommended by Jedlička and Altschuller,[67] is undesirable because undiluted blood yields preparations too thick for accurate thrombocyte enumeration. Pratt,[26] Reid,[31] Sooy and Laurens[68] and Cumings[69] in their indirect methods, and Kemp and Calhoun,[12, 70] Flössner,[18] Hofmann,[19] Degkwitz,[33, 34] Schenk,[35] Wittkower,[36] Pagniez and Mouzon,[39] Achard and Aynaud,[43] Roskam,[50] Kemp,[71] Kemp, Calhoun and Harris,[72] Laker,[73] Muir,[74] Brodie and Russell,[75] and Determann[76] in their modified indirect methods allowed a drop of blood to fall into the diluting fluid placed on a glass slide or contained in a special vessel. This procedure carries with it considerable error in that the undiluted blood is allowed to come in contact with the surface of the skin and with the external air. My previous method involved this error. Bizzozero,[8, 9] Bannerman,[37] Zeller,[38] Pizzini,[77] Sahli,[78] Fonio,[79] Evans,[80] and Mackay[81] punctured the finger through a drop of diluting fluid; by this procedure obviously only the first drop of blood is available, and this does not yield accurate platelet counts, as has been pointed out before. Steinmaurer[66] and Hittmair[82] produce a superficial puncture without drawing blood, place a drop of the diluting fluid over the wound and by pulling the edges of the puncture apart allow some blood to escape into the overlying drop of diluent. It is not possible to pull the edges of the wound apart without exerting pressure in the neighborhood of the puncture, a procedure involving considerable error in platelet counting. Moreover, the use of the first drop of blood increases this error.

Bizzozero,[8, 9] Bannerman,[37] Steinmaurer,[66] Sahli,[78] Fonio,[79] Mackay,[81] Hittmair,[82] and von Boros and Kalstein[83] mixed the blood and the diluent on the finger. This technic involves a number of inaccuracies. During the process of stirring the mixture there is unavoidable contact of blood and skin with resulting destruction or clumping of platelets. If the blood is flowing freely, as it should if a correct count is to be obtained, the blood and diluting fluid form a very large drop in which it is rather difficult to obtain a uniform distribution of the blood and which frequently rolls off the finger, especially in women in whom the surface at the tip of the finger is small. Furthermore, freely flowing blood mixed with only one drop of preserving fluid yields preparations too thick for accurate platelet counting. Dameshek[13] does not stir the blood-diluent mixture at all; he places a drop of the preserving fluid over the puncture wound after the first drop or two of blood has been wiped away, then allows some blood to escape into the overlying drop of diluent and by applying a cover slip to the mixture, carries some of it away. This procedure is inaccurate because the platelets, being very light, quickly rise to the surface of the drop of diluting fluid before the considerably heavier erythrocytes have become uniformly distributed. The fluid on the cover slip, therefore, contains a relatively larger number of platelets than red cells.

The following procedure, in my opinion, obviates all the above mentioned inaccuracies in technic. The palmar surface of the finger tip is prepared in the manner previously described. A puncture is made sufficiently deep to obtain a

fairly free flow of blood without applying pressure. The first drop or two of blood is wiped away; if the blood should form a dry layer on the surface of the skin, it may be washed off with a little of the diluent. A drop of the diluting fluid is then placed over the puncture wound before the blood reaches the surface of the skin and the hand quickly turned over so that the palmar surface is directed downward. The blood, having a greater specific gravity than the diluting fluid, falls rapidly to the bottom to the drop of diluent, as shown in Fig. 1. After a sufficiently large drop of blood has escaped, the entire mixture

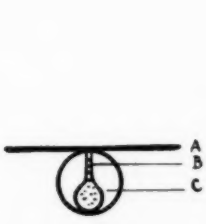


Fig. 1.

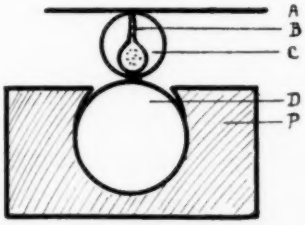


Fig. 2.

Fig. 1.—Technic for initial dilution of blood.

Fig. 2.—Technic for final dilution of blood. *A*, palmar surface of finger; *B*, drop of blood; *C*, drop of diluent; *D*, diluent in paraffin cup; *P*, paraffin cup.

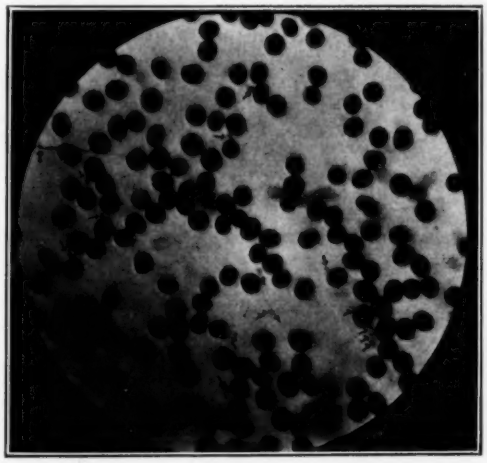


Fig. 3.—Microphotograph of a wet preparation from a normal blood (×800) showing the ideal proportion of erythrocytes and platelets (the latter marked by arrows).

is applied to the surface of a small quantity (three to four drops) of diluting fluid contained in a paraffin cup, as shown in Fig. 2. The entire drop on the finger consisting of approximately equal parts of blood and diluent drops off into the cup. In this manner the blood is diluted approximately 1:5. The cup is prepared by melting the center of a small cube of paraffin, about 2 cm. on the side, with the heated end of a glass rod. The contents of the cup are then stirred gently with a wooden applicator, the end of which is coated with paraffin. The mixture is allowed to stand for a minute or two, stirred again and then is transferred by means of a clean paraffin-coated applicator to a glass slide;

usually three preparations can be obtained as the fluid in one cup yields three large drops. A cover slip is placed over each drop and after the preparations have been allowed to stand for ten to fifteen minutes, a relative platelet erythrocyte count is made, using the oil immersion lens. The glassware must be scrupulously clean, for, as Fenn[84] showed, hemolysis is most marked on slightly soiled glassware and may occur so rapidly with unfixed or improperly fixed cells that accurate counts are rendered impossible. When hemolysis occurs, artefacts in the form of "Arnold bodies" nearly always appear. Each oil immersion field should contain 100 to 150 erythrocytes (Fig. 3). In such a preparation the platelets appear as clear, isolated, highly refractile bodies suspended in the fluid medium, and are either motionless or exhibit Brownian motion. In thin preparations both the platelets and erythrocytes are liable to trauma from pressure of the cover slip; moreover, the platelets are often seen clinging to the underlying glass slide and eventually losing their refractile appearance, spreading out and disintegrating into coarse granules easily mistaken for small platelets. This explains the unduly high platelet counts frequently obtained in very thin preparations. In thick preparations the red cells often cover some of the platelets which escape the count. The platelets and erythrocytes are both counted in fields taken at random in different parts of the three preparations, until at least 1,000 red cells have been seen. An erythrocyte count is then done in the usual manner, and the absolute number of platelets per cubic millimeter is determined.

Table I represents the platelet counts obtained by the method described here in thirty-eight normal persons, twenty-two males and sixteen females.

TABLE I

NORMAL PLATELET COUNTS BY THE AUTHOR'S METHOD

MALES		FEMALES	
AGE	PLATELETS PER C.MM.	AGE	PLATELETS PER C.MM.
35	494,000	40	470,000
27	521,000	40	482,000
28	500,000	24	491,000
40	568,000	30	480,000
33	500,000	41	541,000
31	505,000	40	512,000
25	570,000	52	437,000
33	495,000	45	559,000
33	516,000	20	474,000
26	511,000	40	513,000
35	514,000	19	445,000
30	488,000	16	528,000
35	502,000	20	500,000
24	486,000	22	538,000
35	570,000	40	564,000
32	577,000	52	519,000
14	502,000		
38	581,000		
28	546,000		
32	552,000		
44	510,000		
30	540,000		

The minimum count for the group was 437,000 platelets per cubic millimeter; the maximum, 586,000. The average for the group was 514,000 platelets per cubic millimeter; the average for males was 525,000; that for females was 504,000.

In order to test the accuracy of the method, platelet counts were done simultaneously from two different fingers of the same person. The results are given in Table II.

The number of thrombocytes determined under the same physiologic conditions at the same hour of the day remains fairly constant. This is shown in Table III which represents the comparative platelet counts obtained from the same person on different days.

TABLE II

COMPARISON OF PLATELET COUNTS FROM TWO DIFFERENT FINGERS ON THE SAME PERSON

SEX	AGE	PLATELETS PER C.MM. FINGER I	FINGER II	PERCENTAGE OF VARIATION	REMARKS
M	35	434,000	436,000	0.4	Case of pernicious anemia under liver treatment
F	31	439,000	438,000	0.2	Chronic secondary anemia
F	33	637,000	635,000	0.2	Polycythemia
F	40	557,000	543,000	2.5	Catarrhal jaundice
F	16	580,000	590,000	1.7	Bronchial asthma
F	32	450,000	428,000	5.0	Chronic secondary anemia
M	35	565,000	575,000	1.8	Normal
M	32	519,000	536,000	3.2	Normal
F	40	564,000	563,000	0.2	Normal
M	35	517,000	511,000	1.1	Normal
M	28	500,000	495,000	1.0	Normal
F	41	547,000	545,000	0.4	Normal
M	25	583,000	558,000	4.4	Normal
M	33	504,000	496,000	1.6	Normal
M	45	537,000	505,000	6.1	Normal
M	33	482,000	494,000	2.7	Normal

TABLE III

VARIATION OF THE PLATELET COUNT IN ADULTS

SEX	AGE	DATE (1933)	PLATELETS PER C.MM.	AVERAGE	PERCENTAGE OF VARIATION	REMARKS
F	20	Oct. 19	451,000			
		Oct. 20	474,000	462,000	5.0	Normal
M	40	Nov. 16	452,000			
		Nov. 17	467,000	459,000	3.0	Mild chronic secondary anemia
M	36	Nov. 3	502,000			
		Dec. 23	494,000	498,000	1.6	Normal
M	37	Oct. 5	581,000			
		Dec. 21	560,000	570,000	3.7	Normal
M	34	Oct. 21	495,000			
		Dec. 19	480,000	487,000	3.0	Normal
M	25	Oct. 26	571,000			
		Dec. 15	578,000	574,000	1.2	Normal
F	22	Sept. 26	538,000			
		Dec. 21	508,000	523,000	5.7	Normal

DISCUSSION OF METHODS EMPLOYED FOR COUNTING THE BLOOD PLATELETS

The numerical estimation of the blood platelets has not come into general clinical practice apparently because simple methods of estimation are not suf-

ficiently accurate and because the more exact methods are not sufficiently simple. The great number of methods and modifications of methods found in the literature indicates a lack of standardization in technic. The methods thus far introduced for enumerating the blood platelets can be divided into three groups: direct methods, indirect methods, and special methods.

Direct Methods.—The direct methods for counting blood platelets involve the use of the graduated pipette and the counting chamber. The results obtained by various investigators employing this method are given in Table IV.

That the direct methods are untrustworthy is shown by the normal platelet counts reported which vary from 100,000 to 1,000,000 per c.mm. Many early investigators (Laker,[73] Muir,[74] Brodie and Russell,[75] Determann,[76] Pizzini,[77] Halla,[85] Rabl[86]) recognized the fallacies of the direct methods. Krumbhaar[87] enumerates sixteen common errors in counting erythrocytes. Everyone of these

TABLE IV

AVERAGE NUMBER OF PLATELETS PER CUBIC MILLIMETER AS DETERMINED BY VARIOUS AUTHORS USING THE DIRECT METHOD

AVERAGE NUMBER OF PLATELETS PER C.MM.	AUTHOR	AVERAGE NUMBER OF PLATELETS PER C.MM.	AUTHOR
100,000 – 350,000	Oestreich[140]	297,000	Wright and Kinnicutt[145]
180,000 – 250,000	Fusari[141]		
210,000	Aynaud[142]	200,000 – 300,000	Afanassiew[146]
228,000	Helber[25]	250,000 – 300,000	Howlett[147]
230,000 – 240,000	Port and Akiyama[143]	300,000	Goadby[148]
245,000	Gutstein[48]	337,000 – 340,000	van Herwerden[149]
245,000	van Emden[73]	300,000 – 500,000	Jepsen[103]
250,000	Hayem[88]	320,000 – 450,000	Preiss[49]
250,000	Gottlieb[29]	360,000 – 500,000	Barbieri[150]
251,000	Maixner and Decastello[32]	500,000	Prus[151]
		536,000	Casey and Helmer[152]
255,000	Ottenberg and Rosenthal[144]	600,000	Puchberger[153]
		1,000,000	Lampert[91]
284,000	Buckman, and Hallisey[46]		

errors is possible also when counting platelets by the direct methods in addition to the following: Clumping of the platelets during the drawing of the blood into the pipette, the impossibility of visualizing the smaller platelets with the high dry lens, the failure to visualize some of the platelets in the 100 microns space that exists between the bottom of the counting chamber and the overlying cover glass, the sticking of the platelets to the sides of the pipette and to the parts of the hemacytometer, and the confusion of foreign particles or precipitates with platelets. Even the temperature of the pipette in this method is apt to cause considerable error, as was pointed out by Kemp, Calhoun and Harris[72] and by Hayem;[88] in a warm room the error would be greater than in a cold room. A source of error frequently overlooked is the dilution of the blood. Windfeld[89, 90] showed that plasma diluted 1:20 will often give platelet counts twice as great as undiluted plasma, due to the fact that in higher dilutions the platelets disintegrate readily, yielding greater values. This is especially liable to occur when diluents are employed which preserve the platelets poorly, as Tyrode's solution. This source of error explains partly the extremely high normal values reported by Lampert[91] who employed a specially constructed pipette made of

amber. The direct methods for counting blood platelets, therefore, with twenty-three possible sources of error, are totally unreliable.

Indirect Methods.—In the indirect methods either the graduated pipette alone, or both the pipette and counting chamber are dispensed with. The ratio of platelets to red cells is first obtained. Then, by doing an erythrocyte count in the usual manner, the absolute number of platelets per cubic millimeter is calculated. Three different indirect methods are being employed for platelet counting.

1. *Dry Indirect Methods:* Dried films are prepared from undiluted or diluted blood, stained with some suitable dye and the ratio of platelets to erythrocytes determined. Introduced by Rabl[86] in 1896, it was employed with some modifications by Steinmaurer,[66] Fonio,[79] Hittmair,[82, 92] Gottlieb,[93] Stahl,[94] Oselladore,[95] Leschke and Wittkower,[96] Gáspár[97] and Doenecke.[98] With the exception of Hittmair and Steinmaurer, the investigators using this method obtained normal counts of approximately 250,000 platelets per cubic millimeter. This method is not trustworthy; many of the platelets are destroyed during the preparation of the films, and some are washed off during the process of staining. Hittmair's modification of this method yields normal counts of about 480,000 platelets per cubic millimeter; that of Steinmaurer, 690,000. Some of the inaccuracies involved in the technic of these two investigators have been pointed out before. Moreover, Hittmair employs as diluent a 14 per cent solution of magnesium sulphate which is highly hypertonic and, according to Boshamer,[99] breaks up some of the platelets, thus raising the count. Steinmaurer used Tyrode's solution as the diluting fluid; the errors involved when this solution is employed as a preservative for platelets will be discussed later. Apparently the dry indirect methods, although simple in their application, possess many inherent sources of error and are, therefore, unreliable.

2. *Modified Indirect Methods:* In these methods the graduated pipette is discarded, the blood being mixed with the diluent on the finger, on a glass slide, or in a special vessel, and a drop of the mixture then placed in a counting chamber where the relative number of platelets and erythrocytes is determined. The results obtained by various investigators using this method are given in Table V.

TABLE V

AVERAGE NUMBER OF BLOOD PLATELETS PER CUBIC MILLIMETER OF NORMAL BLOOD AS DETERMINED BY VARIOUS AUTHORS USING THE MODIFIED INDIRECT METHOD

AVERAGE NUMBER OF PLATELETS PER C.MM.	AUTHOR	AVERAGE NUMBER OF PLATELETS PER C.MM.	AUTHOR
150,000 – 200,000	Sahli[78]	300,000	Evans[80]
210,000 – 245,000	Roskam[50]	300,000	Degkwitz[33, 34]
216,000	Achard and Aynaud[43]	400,000	Laker[73]
		590,000 – 760,000	Cumings[69]
200,000 – 250,000	Muir[74]	635,000	Brodie and Russell[75]
227,000	Determann[76]	682,000 – 760,000	Flössner,[18] Hofmann[19]
230,000	Schenk[35]		
250,000	Bizzozero[8]	600,000 – 900,000	Horwitz[41]
238,000 – 388,000	Pagniez and Mouzon[39]	778,000 – 862,000	Kemp and Calhoun[12, 70]
250,000 – 300,000	Wittkower[36]		

In the ordinary hemacytometer foreign bodies might be easily confused with platelets; hence the unreliability of counts obtained in this manner. Even when the dry high power lens is used, products of degenerated erythrocytes ("Arnold bodies"), foreign particles and bacteria may be mistaken for platelets. The platelets themselves, particularly the small forms, are often recognized with difficulty in the chamber. A serious objection to the use of the counting chamber is its depth, as was pointed out by Pratt,[26] Halla,[85] and others. The ordinary Thoma-Zeiss hemacytometer is 0.1 mm. or 100 microns deep, which is about forty times the diameter of the average platelet. The erythrocytes quickly settle to the bottom, but the platelets, being considerably lighter in weight, tend to float in the upper layers of the fluid and settle to the bottom very slowly. It is, therefore, difficult not to miss some of the platelets in the count. Even when specially constructed chambers with smaller depths are employed, methods involving their use are unreliable (Helber,[25] Pratt[26]). The counts obtained by the modified indirect methods, therefore, will below if many of the platelets escape the count because of the impossibility of visualizing them all in the hemacytometer; or the counts will be too high if foreign particles and bacteria in the counting chamber are mistaken for platelets. Kemp and Calhoun's[12, 70] high counts, for example, are due to the inclusion in the platelet counts of "Arnold bodies" which are not readily differentiated from platelets with the dry high power lens.

The modified indirect method of Flössner[18] and Hofmann[19] where Tyrode's solution is used as the diluting fluid, has been adopted recently by a number of investigators (Horwitz,[41] Cumings,[69] Boshamer,[99] Kolozs,[100] Schulte[101] and Weiss[102]). Tyrode's solution is also the diluting fluid in the direct methods of Preiss,[49] Lampert,[91] Jepsen,[103] and Behr,[104] and in the dry indirect method of Steinmaurer.[66] This solution, which is a good artificial nourishing fluid, has the following composition: NaCl 8; KCl 0.2; $CaCl_2$ 0.1; NaH_2PO_4 0.05; $NaHCO_3$ 1; dextrose 1; oxygen to saturation, and H_2O 1,000. When Tyrode's solution is used as a diluting fluid, numerous small, rounded bodies, approximately 0.5 to 1 micron in diameter, appear in the preparations. They are very infrequent in other preserving fluids. These bodies exhibit very active Brownian-like motions, changing their position continuously among the red cells. They do not stain readily with the ordinary dyes and appear to be highly resistant when in contact with glass, much more so than platelets. There has been considerable controversy as to the nature of these bodies. Horwitz,[41] Steinmaurer,[66] Cumings,[69] Boshamer,[99] Kolozs,[100] Schulte[101] and Weiss[102] consider them as platelets. Lampert[105] considers them as crystalline products formed by the interaction of the solution with glass, with the formation of $CaCO_3$:

$$CaCl_2 + 2NaHCO_3 = CaH_2(CO_3)_2 + 2NaCl$$
$$CaH_2(CO_3)_2 + 2NaOH = CaCO_3 + Na_2CO_3 + 2H_2O$$

$CaCO_3$, being slightly soluble, is precipitated out in the form of crystals. By preparing a special optically pure Tyrode's solution Lampert was able to reduce the number of these bodies, but there were still many present. They are present even after the solution has been carefully filtered ten to twelve times

(Eufinger and Knobloch[106]). Lampert[105] as well as Eufinger and Knobloch[106] also believe that some of these formations are colloidal bodies. Doenecke,[98] Jepsen,[103] Behr,[104] and Hartmann[107] consider these bodies to be artefacts whose nature is uncertain.

The small bodies above referred to are very similar in appearance and size to the formations described by Edelmann[108] as the fourth blood element and believed by Pelczar and Koloszynski[109] to represent microthrombocytes arising during the process of disintegration of platelets. Moreover, these small bodies are also quite similar, according to Blacher,[110] to hemokonia or blood dust first described by Müller;[111] although best seen when examined in the dark-field, they can at times be observed with the oil immersion lens in ordinary preparations. Hemokonia may arise from fat droplets in the blood (Schilling[112]), from blood platelets (Horwitz,[41] Boshamer[99]), and possibly from white blood sells (Behr[104]). The degeneration products of erythrocytes known as pseudospirochetes and filamentous forms ("Blutfäden") and described by Zeller,[113] Schultz,[114] Takeuchi,[115] von Neergaard,[116] Auer,[117] Andre,[118] and Doerr and Seidenberg,[119] rapidly break up with the formation of coccoid forms similar to hemokonia. This is particularly apt to occur in blood from febrile patients. Furthermore, the above mentioned small bodies are very similar to some of the smaller "Arnold bodies" (degeneration products of red cells) described by Schultze,[5] Osler,[7] Hayem,[88] Wlassow[120] and particularly by Arnold[121] and visible in ordinary oil immersion preparations. These minute bodies, in other words, are either artefacts or fragments of disintegrated platelets or of the other cellular blood constituents. They possess neither the morphology nor the staining characteristics of platelets. They cannot, therefore, be considered as thrombocytes.

A characteristic feature in preparations where Tyrode's solution is employed as the diluent, is the presence of large numbers of small thrombocytes whose size is one-quarter the diameter of a red cell (1.8 microns) or less. Some investigators using this solution (Flössner,[18] Hofmann,[19] Horwitz,[41] Cumings,[69] Boshamer[99]) are of the opinion that in it the smaller platelets are preserved; these small forms, they believe, are very fragile and are apt to disintegrate rapidly, thus escaping the count. Jürgens[42] who employed a modification of Thomsen's[45] method, holds a similar view. In Tyrode's solution the small thrombocytes form 50 to 70 per cent of all of the platelets. The average size of normal platelets as reported by various observers not using Tyrode's solution, is given in Table VI.

TABLE VI

AVERAGE DIAMETER OF PLATELETS AS DETERMINED BY VARIOUS AUTHORS

AVERAGE DIAMETER OF PLATELETS IN MICRONS	AUTHOR	AVERAGE DIAMETER OF PLATELETS IN MICRONS	AUTHOR
0.5 – 2.0	Dameshek[13]	2.0 – 3.0	Mackay[81]
2.0 – 5.0	Richardson[27]	3.0	Stahl[94]
2.0 – 3.0	Degkwitz[33, 34]	2.0 – 6.0	Kolozs[100]
2.0 – 3.0	Achard and Aynaud[43]	2.0 – 5.0	Reimann[132]
2.0 – 3.0	Preiss[49]	2.5 – 4.0	Howlett[154]
2.0 – 4.0	van Emden[73]	1.8 – 3.6	Afanassiew[155]
2.0	Muir[74]	3.0	Maximow and Bloom[156]
2.5 – 5.0	Determann[76]		

From this table it is obvious that the average size of normal platelets, according to most investigators, is 2 microns or greater; Dameshek's[13] smaller figures are due to the fact that his solution is hypertonic.

According to size, platelets are best classified into three groups: Group I, consisting of platelets whose size is one-quarter the diameter of a red cell, or about 1.8 microns; Group II, consisting of platelets one-third the diameter of a red cell, or about 2.5 microns; and Group III, consisting of platelets one-half the diameter of a red cell or greater, or about 3.6 microns. The occasionally present irregular forms may be placed in Group IV. Table VII represents the classification of platelets according to size, as determined in wet preparations by Boshamer,[99] Degkwitz[33] and by the author.

TABLE VII

DIFFERENTIATION OF PLATELETS ACCORDING TO SIZE AS DETERMINED IN WET PREPARATIONS

AUTHOR	GROUP I	GROUP II	GROUP III	GROUP IV	REMARKS
Boshamer	49%	43%	3.5%	4.5%	Tyrode's solution used as diluent
Boshamer	17%	74%	4.0%	5.0%	14% $MgSO_4$ solution used as diluent
Boshamer	18%	72.5%	4.5%	5.0%	Degkwitz's solution used as diluent
Degkwitz	–	94.4%	5.6%	–	Sodium metaphosphate used as diluent
Author	18.8%	72.2%	9.0%	–	Average results obtained on 18 normal persons. One per cent sod. metaphosphate used as diluent

Most platelets, apparently, are medium sized with an average diameter of 2.5 microns. Why is it, then, that when Tyrode's solution is used, a large percentage of the platelets are of the smaller variety, or Group I? The explanation is given by a number of investigators (Preiss,[49] Jepsen,[103] Lampert,[105] Hartmann[107]) who observed that in Tyrode's solution after a relatively short time, twenty to thirty minutes, the smaller platelets increase in number as the larger platelets diminish. Evidently the increase in the number of the smaller platelets is due to the disintegration of the larger forms. Preiss[49] proved this conclusively in his experiments by injecting hirudin into rabbits before drawing blood for platelet counting, in order to prevent extravascular disintegration of the thrombocytes. Although Tyrode's solution, which is a poor preservative for platelets, was employed as the diluting fluid, yet the number of small platelets, even at the end of one hour, remained approximately the same. Preiss,[49] Jepsen,[103] Behr,[104] Lampert[105] and Baedorf[122] who used Tyrode's solution as diluent, believing the diminutive platelets to represent disintegration products, excluded them from their counts.

The Flössner-Hofmann modified indirect method for platelet counting evidently involves errors due to the use of the counting chamber and to the em-

ployment of Tyrode's solution as diluent which contains a variety of artefacts easily confused with platelets. This method for enumerating the thrombocytes is, therefore, untrustworthy.

3. *True Indirect Methods:* In these methods both the graduated pipette and the counting chamber are dispensed with. A drop of the diluted blood is placed on a clean glass slide, covered with a cover slip and by using the oil immersion lens the ratio of platelets and erythrocytes determined. These methods for counting blood platelets are, in my opinion, the most accurate because they involve a minimum manipulation of the blood. The results obtained by various investigators using this method are given in Table VIII.

TABLE VIII

AVERAGE NUMBER OF BLOOD PLATELETS PER CUBIC MILLIMETER AS DETERMINED BY VARIOUS AUTHORS USING THE TRUE INDIRECT METHOD

AVERAGE NUMBER OF PLATELETS PER C.MM.	AUTHOR	AVERAGE NUMBER OF PLATELETS PER C.MM.	AUTHOR
250,000	Bannerman[37]	327,000	Cadwalader[123]
300,000	Reid[31]	390,000	Mackay[31]
312,000	Cramer and Bannerman[47]	469,000	Pratt[26]
		590,000 – 760,000	Cumings[69]
300,000 – 600,000	von Boros and Kalstein[83]	619,000	Olef[11]
		716,000	Dameshek[13]

The considerable divergence in the results reported is due either to inaccuracies in the technic employed, or to the preserving fluid used as diluent. Bannerman's[37] technic is inaccurate because he used the first drop of blood and then mixed the blood and diluent on the finger. The procedures of Reid,[31] Cadwalader[123] and Pratt[26] are very similar and involve errors because the first drop of blood drawn is transferred to a drop of diluent on a glass slide, thus permitting the blood to come in contact with the surface of the skin and the external air. The method of Cramer and Bannerman[47] contains several errors: (1) too great manipulation of the blood which is obtained by venipuncture, (2) too great dilution of the blood (1:10), and (3) mixing blood and diluent with a teated pipette not covered with paraffin. Von Boros and Kalstein[83] as well as Mackay[81] used the first drop of blood and mixed the blood and diluting fluid on the finger; this procedure is inaccurate. Cumings[69] employed Tyrode's solution which is a very poor preserving fluid for platelets. My previous method[11] involved the error of allowing the blood to come in contact with the surface of the skin and the external air. Dameshek's[13] method is inaccurate because, as previously noted, the blood and diluent do not form a uniform mixture, thus yielding relatively high platelet counts.

Special Methods.—Zeller's Method: The method of Zeller[38] is essentially a modified indirect method and is too complicated for clinical purposes, as it involves the use of the torsion balance. By employing Kemp-Calhoun's[12] diluting fluid he obtained normal values of 500,000 to 750,000 platelets per cubic millimeter. The sources of error in this method are (1) too much handling of the blood, (2) the employment of the hemacytometer, and (3) the use of Kemp-

Calhoun's solution which is a poor preserving fluid for both erythrocytes and platelets. "Arnold bodies," resulting from the disintegration of red cells, are readily formed in this fluid as is obvious from Kemp's[124] description of platelets containing hemoglobin. The platelets also disintegrate rapidly as is apparent from Zeller's[38] description of the platelets as varying in size from the diameter of a red cell to a point. This method is not reliable.

Kristenson's Method: The method of Kristenson[40] involves the use of a specially constructed syringe for obtaining venous blood and the subsequent use of the counting chamber. In 1928 he modified his method[125] by using capillary blood and a special graduated pipette. His method was adopted by Hartmann,[107] Baquero Gil,[126] and Lucia and Rickard.[127] Kristenson obtained normal values of 300,000 platelets per c.mm. with his first method, and from 326,000 to 424,000 platelets per c.mm. with his second method. Neither method is trustworthy for obvious reasons. The first is essentially a modified indirect method, the second a direct method. Moreover, the diluting fluid which consists of urea, sodium citrate, corrosive sublimate and cresyl violet is hypertonic and dissolves the erythrocytes (Kristenson,[40] Lucia and Rickard[127]).

Thomsen's Method: Thomsen's[45] method is based on the original observation of Bürker[128] that platelets remain suspended for a long time in undiluted plasma. Blood is obtained by venipuncture into a syringe containing a small amount of sodium citrate and allowed to stand until the red cells have settled. A small quantity of the supernatant plasma is then drawn into a white cell pipette, diluted with some suitable diluent and a platelet count done in the ordinary hemacytometer. By determining the volume of packed red cells the absolute number of platelets per cubic millimeter of blood can be obtained. Thomsen modified his procedure in 1923 by introducing his micromethod.[129, 130, 131] The results obtained by various investigators using this method, or modifications of it, are given in Table IX.

TABLE IX

AVERAGE NUMBER OF BLOOD PLATELETS PER CUBIC MILLIMETER AS DETERMINED BY VARIOUS AUTHORS USING THOMSEN'S METHOD

AVERAGE NUMBER OF PLATELETS PER C.MM.	AUTHOR	AVERAGE NUMBER OF PLATELETS PER C.MM.	AUTHOR
200,000 – 500,000	Gram[139]	348,900	Petri[133, 134]
230,000	Schenk and Spitz[157]	350,000	Reimann[132]
250,000 – 300,000	Thomsen[45]	352,000 – 531,000	Windfeld[89, 90]
270,000	Spitz[158]	650,000	Jürgens[42]
320,000	Nygaard[159]		

Those employing this method assume that the platelets remain uniformly suspended in the plasma even for as long as six hours. If the number of platelets per cubic millimeter is determined in various levels of the plasma at different intervals the results obtained will usually be fairly uniform. This is due to the fact that when a pipette is introduced into the plasma column for the purpose of drawing some of it off for a platelet count, currents are unavoidably produced in the fluid, mixing the lower and upper layers of the column of

plasma which will yield consistent counts. In fact, following the introduction of the pipette into the plasma, the sudden streaming of the very light platelets from the lower levels of the plasma where they have become more numerous, to the upper levels will at times give higher counts in the upper layers even after the blood has been standing for several hours. By employing Reimann's[132] modification of Thomsen's technic and using a 1 per cent solution of sodium metaphosphate as diluent, I carefully removed with a pipette at hourly intervals a small quantity of plasma from the very surface of the plasma layer, taking great care not to produce any currents in the fluid. At the end of two hours the uppermost level of the plasma column contained 474,000 platelets per c.mm. of plasma; at the end of three hours there were 360,000 platelets per c.mm. in the same plasma level, and at the end of four hours there were 344,000 platelets per c.mm. Moreover, at the end of four hours there were 265,000 platelets per c.mm. in the center of the column of sedimented red cells. Apparently the platelets do not remain uniformly suspended in the plasma for several hours, as is assumed by those employing Thomsen's method, but are gradually gravitating to the lower levels of the column of plasma, the rate of sedimentation of the thrombocytes depending, according to Jürgens,[42] on their number, size, and the composition of the plasma. This has also been observed by Baunack[135] who showed that after one to two hours the concentration of the platelets in the lower layers of the plasma column may be from 40 to 50 per cent greater than their concentration in the upper levels. Moreover, even at the end of several hours there are still a good many platelets among the red cells.

According to Petri,[133, 134] Thomsen's macromethod involves an error of 10.4 per cent with undiluted plasma and an error of 21.9 per cent with diluted plasma. Thomsen's micromethod involves an error of 20.3 per cent according to Petri[136, 137] and an error of 8 to 10 per cent according to Als.[138] Petri's improved "centrifuge" modification[133, 134] of Thomsen's method has a minimum error of 7.1 per cent with venous blood and a minimum error of 9.4 per cent with capillary blood. Thomsen[45] found that after centrifuging the blood for ten minutes at 2,000 revolutions per minute there was still 5 per cent plasma among the red cells, and Gram[139] found that after centrifuging the blood for fifteen minutes at 3,000 revolutions per minute there still remained 5 to 10 per cent of plasma among the red cells.

If to the above numerous sources of error are added those inherent in any direct method for platelet counting, then Thomsen's method and its modifications become entirely untrustworthy.

SUMMARY

The most accurate procedures for counting the blood platelets are the true indirect methods. The true indirect method described here is, I believe, accurate and dependable, maximum care having been exercised in carrying out the various technical steps involved. It adheres to the leading principles that must be taken into consideration in counting platelets: (1) avoidance of contact between undiluted blood and skin, external air, glassware or metal, (2) mixing

of freshly drawn blood as quickly as possible with the preserving fluid, and (3) avoidance of all unnecessary manipulations of the blood. It is simple to carry out and can be easily adapted for routine clinical use.

REFERENCES

1. Donné, M. Al.: De l'origine des globules du sang, de leur mode de formation et de leur fin. Compt. rend. Acad. de Sc. **14:** 366, 1842.
2. Zimmermann, G.: Zur Dynamik des Aderlasses, Arch. f. phys. Heilk. **4:** 65, 1845.
3. Virchow, R.: Die Cellularpathologie, page 200; Berlin, Verlag von August Hirschwald, 1858.
4. Zimmermann, G.: Zur Blutkörperchenfrage, Virchows Arch. f. path. Anat. **18:** 221, 1860.
5. Schultze, M.: Ein heizbarer Objecttisch und seine Verwendung bei Untersuchungen des Blutes, Arch. f. mikr. Anat. **1:** 1, 1865.
6. Osler, W.: An Account of Certain Organisms Occurring in the Liquor Sanguinis, Proc. Roy. Soc. Med., London **22:** 391, 1874.
7. Osler, W.: The Third Corpuscle of the Blood, Medical News, Phila. **43:** 701, 1883.
8. Bizzozero, J.: Ueber einen neuen Formbestandtheil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung, Virchows Arch. f. path. Anat. **90:** 261, 1882.
9. Zizzozero, J.: D'un nouval élément morphologique du sang, Arch. ital. de biol. **2:** 345, 1882.
10. Doan, C. A., and Sabin, F. R.: Local Progression With Spontaneous Regression of Tuberculosis in the Bone Marrow of Rabbits, Correlated With a Transitory Anemia and Leucopenia After Intravenous Inoculation, J. Exper. Med. **46:** 315, 1927.
11. Olef, I.: Blood Platelets: An Improved Indirect Method for Their Enumeration, Arch. Int. Med. **46:** 585, 1930.
12. Kemp, G. T., and Calhoun, H.: Enumeration of Blood Platelets: Their Relation and That of the Leucocytes to Blood Coagulation, Brit. M. J. **2:** 1539, 1901.
13. Dameshek, W.: A Method for the Simultaneous Enumeration of Blood Platelets and Reticulocytes, Arch. Int. Med. **50:** 579, 1932.
14. Rees, H. M., and Ecker, E. E.: An Improved Method for Counting Blood Platelets, J. A. M. A. **80:** 621, 1923.
15. Hédon, E., and Fleig, C.: Action des sérums artificiels et du sérum sanguin sur le fonctionnement des organes isolés des mammifères, Arch. internat. de Phys. 3: 95, 1905, 1906.
16. Adler, H. M.: Experiments Toward a Physiologically Isotonic Solution of Salts, J. A. M. A. **51:** 752, 1908.
17. Tyrode, M. V.: The Mode of Action of Some Purgative Salts, Arch. internat. de pharmacol. et de therap. **20:** 205, 1910.
18. Flössner, O.: Beobachtungen und Zählung von Blutplättchen, Ztschr. f. Biol. **77:** 113, 1922, 1923.
19. Hofmann, F. B.: Ueber Blutplättchenzählung, Deutsche med. Wchnsch. **52:** 861, 1926.
20. Pratt, J. H.: Beobachtungen über die Gerinnungszeit des Blutes und die Blutplättchen, Arch. f. exper. Path. u. Pharmakol. **49:** 299, 1902, 1903.
21. Deetjen, H.: Untersuchungen über die Blutplättchen, Virchows Arch. f. path. Anat. **164:** 239, 1901.
22. Deetjen, H.: Zerfall und Leben der Blutplättchen, Ztschr. f. physiol. Chem. **63:** 1, 1909.
23. Hirschfeld, H.: Ueber die Entstehung der Blutplättchen, Virchows Arch. f. path. Anat. **166:** 195, 1901.
24. Sabbatani, L.: Fonction biologique du calcium, Arch. ital. de biol. **39:** 333, 1903.
25. Helber, E.: Ueber die Zählung der Blutplättchen im Blute des Menschen und ihr Verhalten bei pathologischen Zuständen, Deutsche Arch. f. klin Med. **81:** 316, 1904.
26. Pratt, J. H.: A Critical Study of the Various Methods Employed for Enumeration of Blood Platelets, J. A. M. A. **45:** 1999, 1905.
27. Richardson, F. L.: Effect of Severe Hemorrhage on the Number of Blood Plates in Blood From the Peripheral Circulation of Rabbits, J. Med. Res. **8:** 99, 1904, 1905.
28. Cadwalader, W. B.: A Comparative Study of the Methods of Counting Blood Platelets, Bull. Ayer Clin. Lab. Penn. Hosp. **3:** 50, 1906.
29. Gottlieb, I.: Zur Frage der Blutplättchen, Folia haemat. **5:** 447, 1908.
30. Aynaud, M.: Action du metaphosphate de soude sur les globulins, Compt. rend. Soc. de biol. **68:** 916, 1910.
31. Reid, H. S.: Methods of Examining and Enumerating Blood Plates, Edinb. M. J. **8:** 432, 1912.
32. Maixner, E., and von Decastello, A.: Klinische Untersuchungen über das gegenseitige Verhältnis der Leukocyten—und Blutplättchenzahlen, Med. Klin. **11:** 14, 1915.
33. Degkwitz, R.: Studien über Blutplättchen, Folia haemat. **25:** 153, 1919, 1920.
34. Degkwitz, R.: In Abderhalden's Handbuch der Biologischen Arbeitsmethoden; Berlin, 1924, Urban u. Schwarzenberg.

35. Schenk, M.: Ein Verfahren zum Zählen du Blutplättchen, München. med. Wchnschr. **68**: 427, 1921.
36. Wittkower, E.: Klinische und experimentelle Untersuchungen zur Blutplättchenfrage, Ztschr. f. d. ges. exper. Med. **25**: 73, 1921.
37. Bannerman, R. G.: Blood Platelet Counting in Man, Lancet **1**: 1154, 1923.
38. Zeller, H.: Neue Methode der Blutplättchenzählung nebst einigen Resultaten, Ztschr. f. d. ges. exper. Med. **10**: 103, 1919, 1920.
39. Pagniez, P., et Mouzon, J.: Procédé de numération des plaquettes du sang, Compt. rend. Soc. de biol. **85**: 157, 1921.
40. Kristenson, A.: A New Method for the Direct Counting of the So-called Blood Platelets in Man, Acta med. Scandinav. **57**: 301, 1922, 1923.
41. Horwitz, S.: Zur Frage der physiologischen Thrombocytenzahl, Klin. Wchnschr. **12**: 705, 1933.
42. Jürgens, R.: Eine Methode zur Thrombocytenzählung, Klin. Wchnschr. **13**: 97, 1934.
43. Achard, C., et Aynaud, M.: Sur l'observation directe des hémoblastes dans le plasma sanguin, Compt. rend. Soc. de biol. **63**: 593, 1907.
44. Achard, C., et Aynaud, M.: Les globulins dans les infections par les protozoaires, Compt. rend. Soc. de biol. **67**: 213, 1909.
45. Thomsen, O.: En Metode til direkte Taelling af Blodpladerne i Blodet, Hospitalstid. **62**: 161, 1921.
46. Buckman, T. E., and Hallisey, J. H.: Studies in the Properties of Blood Platelets, J. A. M. A. **76**: 427, 1921.
47. Cramer, W., and Bannerman, R. G.: The Clinical Significance of Blood Platelets, Lancet **1**: 992, 1929.
48. Gutstein, M.: Ueber eine neue Methode zur Zählung der Blutplättchen in der Zählkammer, Med. Klin. **28**: 1277, 1932.
49. Preiss, W.: Ueber die physiologische Zahl und Morphologie der Blutplättchen, Ztschr. f. d. ges. exper. Med. **84**: 810, 1932.
50. Roskam, J.: Contribution à l'étude de la physiologie normale et pathologique du globulin, Arch. internat. de physiol. **20**: 241, 1922, 1923.
51. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, ed. 5, Berlin, 1931, Julius Springer.
52. Morawitz, P., and Brugsch, H.: Grundsätzliches über Blutplättchenagglutination, Arch. f. exper. Path. u. Pharmakol. **172**: 657, 1933.
53. Starlinger, W., and Sametnik, S.: Ueber die Entstehungsbedingungen der Thrombose, Verhandl. d. deutsch. Gesellsch. f. inn. Med. **39**: 152, 1927.
54. Stuber, B., und Lang, K.: Zur Pathogenese und Therapie der Thrombose, Klin. Wchnschr. **9**: 1113, 1930.
55. Dietrich: Thrombose, Ihre Grundlagen und Ihre Bedeutung, Berlin, Julius Springer, 1932. Cited by Morawitz and Brugsch.[52]
56. Aynaud, M. N.: Le globulin des mammifères, Med. diss., Paris, G. Steinheil, 1909.
57. Roskam, J.: Physiologie normale et pathologique du globulin (plaquette de Bizzozero), in les problèmes biologiques, Paris, Les Presses Univ. de France, 1927.
58. Govaerts, P.: Le role des plaquettes sanguines dans l'immunité naturelle, Compt. rend. Soc. de biol. **82**: 927, 1919.
59. Govaerts, P.: Intervention des opsonines dans le phénomène d'accolement des microbes aux plaquettes sanguines, Compt. rend. Soc. de biol., Paris **83**: 196 (Jan. 31), 1920.
60. Govaerts, P.: Technique de l'étude "in vitro" de l'accolement des microbes aux plaquettes sanguines, Compt. rend. Soc. de biol. **83**: 197, 1920.
61. Govaerts, P.: Étude de l'accolement "in vitro" des plaquettes sanguines aux globules rouges éstrangers, Compt. rend. Soc. de biol. **83**: 1232, 1920.
62. Osterhout, W. J. C.: Some Aspects of Cell Physiology, Ann. Int. Med. **7**: 396, 1933.
63. Pemberton, R.: The Etiology and Pathology of Arthritis, J. A. M. A. **87**: 1253, 1926.
64. Pierce, E. G., and Pemberton, R.: The Red Cell Count in Arthritis, Arch. Int. Med. **39**: 421, 1927.
65. Crouter, C. Y., and Cajori, F. A.: The Red Cell Count in Arthritis, Arch. Int. Med. **39**: 429, 1927.
66. Steinmaurer, H. J.: Zur Frage der Blutplättchenzahlen und ihrer Veränderungen unter dem Einflusse des vegetativen Nervensystems, Folia haemat. **46**: 269, 1932.
67. Jedlička, V., and Altschuller, G.: Chronic Essential Thrombopenia, Acta med. Scandinav. **64**: 123, 1926.
68. Sooy, J. W., and Laurens, H.: A Method for Counting Blood Platelets in the Rat, Proc. Soc. Exper. Biol. & Med. **22**: 116, 1924.
69. Cumings, J. N.: A Method for Enumeration of Blood Platelets, Lancet **1**: 1230, 1933.
70. Kemp, G. T., and Calhoun, H.: Some New Observations on Blood Plates and Leucocytes, Am. J. Physiol. **5**: 4, 1901.
71. Kemp, G. T.: Relation of Blood Plates to the Increase in Number of Red Corpuscles at High Altitudes, Am. J. Physiol. **6**: 11, 1902.

72. Kemp, G. T., Calhoun, H., and Harris, C. E.: The Blood Plates, Their Enumeration in Physiology and Pathology, J. A. M. A. **46**: 1022, 1906.
73. Laker: Zitzungsschericht der Akad. der Wissenschaft, Wien. vol. cxiii, 1886. Cited by van Emden, J. E. G.: Klinische Untersuchungen über die Blutplättchen, Fortschr. d. Med. **16**: 241, 1898.
74. Muir, R.: Contributions to the Physiology and Pathology of the Blood, J. Anat. & Physiol. **25**: 256, 1891.
75. Brodie, T. G., and Russell, A. E.: The Enumeration of Blood Platelets, J. Physiol. 21: 390, 1897.
76. Determann, D.: Klinische Untersuchungen über Blutplättchen, Deutsche Arch. f. klin. Med. **61**: 365, 1898.
77. Pizzini: Reforma Medica, ii, 1894, cited by van Emden.[73]
78. Sahli, H.: Ueber das Wesen der Hämophilie, Ztschr. f. klin. Med. **56**: 264, 1905.
79. Fonio, A.: Ueber ein neues verfahren der Blutplättchenzählung, Deutsche Ztschr. f. Chir. **117**: 176, 1912.
80. Evans, W. H.: The Blood Changes After Splenectomy in Splenic Anemia, Purpura Haemorrhagica and Acholuric Jaundice, With Special Reference to Platelets and Coagulation, J. Path. & Bact. **31**: 815, 1928.
81. Mackay, W.: The Blood Platelet: Its Clinical Significance, Quart. J. Med. **24**: 285, 1931.
82. Hittmair, A.: Die Blutplättchen, Folia haemat. **35**: 156, 1928.
83. Von Boros, J., and Kalstein, O.: Ein Beitrag zur Frage der Blutplättchenzählung, Folia haemat. **35**: 419, 1928.
84. Fenn, W. O.: Hemolysis of Erythrocytes in Contact With Glass, J. Exper. Med. 35: 271, 1922.
85. Halla, A.: Ueber den Haemoglobingehalt des Blutes und die quantitaven Verhältnisse der rothen und weissen Blutkörperchen bei acuten fieberhaften Krankheiten, Ztschr. f. Heilk. **4**: 331, 1883.
86. Rabl, H.: Ueber eine elective Färbung der Blutplättchen in Trochenpräparaten, Wien. klin. Wchnschr. **9**: 1060, 1896.
87. Krumbhaar, E. B. in Cowdry, E. V.: Special Cytology, New York, P. B. Hoeber, Inc., 1928.
88. Hayem, G.: Du Sang, Paris, G. Masson, 1889.
89. Windfeld, P.: Ueber Blutplättchenzählung, Acta med. Scandinav. **73**: 10, 1930.
90. Windfeld, P.: Zählung der Blutplättchen, Hospitalstid. **73**: 85, 1930.
91. Lampert, H.: Die Bedeutung von Athrombit (echt Bernstein) fur die Thrombocytenzählung, Verhandl. d. deutsch. Gesellsch. f. inn. Med. **43**: 92, 1931.
92. Hittmair, A. in Hirschfeld, H. and Hittmair, A.: Handbuch der allgemeinen Hämotologie, Berlin, Urban u. Schwarzenberg, 1932.
93. Gottlieb, D.: Zur Frage der Blutplättchen, Folia haemat. **5**: 447, 1908.
94. Stahl, R.: Ueber die Notwendigkeit prinzipieller Berücksichtigung der Blutplättchen bei Klinischen Blutuntersuchungen, München. med. Wchnschr. **68**: 667, 1921.
95. Oselladore, G.: Eine Methode zur histologischen Untersuchung und Zählung der Blutplättchen, Ztschr. f. wissensch. Mikr. **42**: 415, 1925.
96. Leschke, E., and Wittkower, E.: Die Werlhofsche Blutfleckenkrankheit, Ztschr. f. klin. Med. **102**: 649, 1925, 1926.
97. Gáspár, S.: Untersuchungen über Ursprung, Zahl und Form der Blutplättchen und über das Benehmen der Knochenmarksriesenzellen (Megakaryozyten) unter normalen und pathologischen Verhältnissen, Frankf. Ztschr. f. Path. **34**: 460, 1926.
98. Doenecke, F.: Ueber die Veränderungen der Blutplättchenzahlen bei akuter diffuser Glomerulonephritis und ihre Bedeutung für die Pathogenese, Ztschr. f. klin. Med. **121**: 121, 1932.
99. Boshamer, K.: Ueber Zählung, Resistenz und Neubildung von Blutplättchen, Ztschr. f. d. ges. exper. Med. **48**: 631, 1925, 1926.
100. Kolozs, E.: Ueber neuere Thrombozyten—Zählmethoden, Wien. klin. Wchnschr. **41**: 1342, 1928.
101. Schulte, H. J.: Ueber die Zahl der Blutplättchen bei gynäkologischen Erkrankungen währen der Schwangerschaft und im Wochenbett, Zentralbl. f. Gynäk. **53**: 2822, 1929.
102. Weiss, H.: Atropinversuche, Bestimmungen der Blutkörpersenkungsgeschwindigkeit und Thrombozytenzählungen an Typhuskranken der Wehlauer Epidemie, Deutsches Arch. f. klin. Med. **165**: 116, 1929.
103. Jepsen, A.: Klinische Untersuchungen über Thrombocyten, Ztschr. f. klin. Med. **122**: 680, 1932.
104. Behr, C. H.: Vergleichende Zählungen der Thrombocyten nach verschiedenen Zählmethoden, Klin. Wchnschr. **12**: 1771, 1933.

105. Lampert, H.: Weitere Erfahrungen mit der Bernsteinmethode der Thrombocytenzählung, Deutsche med. Wchnschr. **57**: 2016, 1931.
106. Eufinger, H., and Knobloch, K.: Das Verhalten von Thrombocytenzahl und Gerinnungszeit währen der Gestationsperiode, Arch. f. Gynäk. **149**: 391, 1932.
107. Hartmann, E.: Ueber den Wert der Hofmann-Flössnerschen Methode der Blutplättchenzählung, Klin. Wchnschr. **11**: 1828, 1932.
108. Edelmann, A.: Ueber ein bisher unbekanntes Blutelement, Wien. klin. Wchnschr. **44**: 795, 1931.
109. Pelczar, K., and Koloszynski, E.: Ueber ein bisher unbekanntes Blutelement, Wien. klin. Wchnschr. **44**: 1114, 1931.
110. Blacher, L.: Hämokonien, neue mit Eigenbewegung gegabte Blutelemente, Polskie Arch. med wewn. **10**: 343, 1932; abst. Kongresszentralbl. f. d. ges. inn. Med. **69**: 660, 1933.
111. Müller, H. F.: Ueber einen bisher nicht beachteten Formbestandtheil des Blutes, Centralbl. f. allg. Path. u. path. Anat. **7**: 529, 1896.
112. Schilling, V.: The Blood Picture and Its Clinical Significance, Trans. by R. B. H. Gradwohl, St. Louis, 1929, The C. V. Mosby Co.
113. Zeller, H.: Die Differenzierung der Blutplättchen, Deutsche med. Wchnschr. **47**: 505, 1921.
114. Schultz, E. W.: The Pseudospirochetes Derived From Red Blood Cells, J. Lab. & Clin. Med. **8**: 375, 1923.
115. Takeuchi, K.: Untersuchungen über Blutfäden, Folia haemat. **34**: 259, 1926, 1927.
116. Von Neergaard, K.: Zur Genese gewisser ätiologisch bisher unbekannter Erkrankungen, Klin. Wchnschr. **11**: 1661, 1932.
117. Auer, J.: The Formation of Macrocytes and Microcytes From Red Corpuscles in Hanging Drop Preparations, J. Exper. Med. **56**: 551, 1932.
118. Andre, H.: Pseudospirochäten in Blutpräparaten, Klin. Wchnschr. **11**: 1918, 1932.
119. Doerr, R., and Seidenberg, S.: Extravasculäre Veränderungen von Erythrocyten, Klin. Wchnschr. **12**: 227, 1933.
120. Wlassow, K.: Untersuchungen über die histologischen Vorgänge bei der Gerinnung und Thrombose mit besonderer Berücksichtung der Entstehung der Blutplättchen, Ziegler's Beitrage z. path. Anat. u. allg. Path. **15**: 543, 1894.
121. Arnold, J.: Zur Morphologie und Biologie der rothen Blutkörper, Virchows Arch. f. path. Anat. **145**: 1, 1896.
122. Baedorf, K.: Kritische Betrachtungen über die Methodik der Thrombozytenzählung, Deutsche med. Wchnschr. **59**: 1643, 1933.
123. Cadwalader, W. B.: A Comparative Study of the Methods of Counting Blood Platelets, Bull. Ayer Clin. Lab. Penn. Hosp. **3**: 50, 1906.
124. Kemp, G. T.: The Effect of Altitude on Blood Plates and Blood Corpuscles, Bull. Johns Hopkins Hosp. **15**: 177, 1904.
125. Kristenson, A.: Zur Methodik der Thrombocytenzählung beim Menschen, Acta med. Scandinav. **69**: 227, 1928.
126. Baquero Gil, G.: Modificaciones del metodo de Kristenson para el recuento de plaquetas, Arch. cardiol. y. hemat. **11**: 140, 1930.
127. Lucia, S. P., and Rickard, J. F.: Effect of Massage on Blood Platelet Production, Proc. Soc. Exper. Biol. & Med. **31**: 87, 1933.
128. Bürker, K.: Eine einfache Methode zur Gewinnung von Blutplättchen, Centralbl. f. Physiol. **17**: 137, 1903.
129. Thomsen, O.: En Mikrometode til Taelling af Blodplader, Hospitalstid. **66**: 406, 1923.
130. Thomsen, O.: Micro-méthode pour la numération des plaquettes dans le sang, Compt. rend. Soc. de biol. **89**: 800, 1923.
131. Thomsen, O.: A Micromethod for the Counting of Blood Plates, Acta med. Scandinav. **58**: 515, 1923.
132. Reimann, H. A.: The Blood Platelets in Pneumococcus Infections, J. Exper. Med. **40**: 553, 1924.
133. Petri, S.: A New Method for Counting Blood Platelets in Undiluted Citrated Plasma, J. Lab. & Clin. Med. **14**: 950, 1929.
134. Petri, S.: Sur la numération des hémoblastes d'après la méthode de Thomsen, Compt. rend. Soc. de biol. **91**: 405, 1924.
135. Baunack: Inaug. Diss., Leipzig, 1933, cited by Jürgens.[42]
136. Petri, S.: Sur la technique employée pour la numération des hémoblastes d'après la méthode de Thomsen dans les recherches expérimentales sur les animaux, Compt. rend. Soc. de biol. **90**: 881, 1924.
137. Petri, S.: Om Tekniken ved Blodpladetaelling ad mod. Thomsen ved Dyreforsøg, Hospitalstid. **67**: 65, 1924.
138. Als, E.: O. Thomsen's Mikrometode til Taelling af Blodplader, Hospitalstid. **66**: 425, 1923.
139. Gram, H. C.: On the Platelet Count and Bleeding Time in Diseases of the Blood, Arch. Int. Med. **25**: 325, 1920.

140. Oestreich, C.: München. med. Wchnschr. **79**: 1794, 1932.
141. Fusari, R.: Arch. per le sc. med. **10**: 235, 1886; Abst. Zentralbl. f. klin. Med. **8**: 45, 1887.
142. Aynaud, M.: In Traité du. Sang by Gilbert, A., and Weinberg, M., Paris, 1913, J. B. Bailliere et Fils.
143. Port, D., and Akiyama, D.: Deutsches Arch. f. klin. Med. **106**: 362, 1912.
144. Ottenberg, R., and Rosenthal, N.: J. A. M. A. **69**: 999, 1917.
145. Wright, J. H., and Kinnicutt, R.: J. A. M. A. **56**: 1457, 1911.
146. Afanassiew, M.: Deutsches Arch. f. klin. Med. **35**: 217, 1884.
147. Howlett, E. H.: Lancet **1**: 223, 1882.
148. Goadby, H. K.: J. Path. & Bact. **33**: 1171, 1930.
149. Van Herwerden, M. A.: Nederl Tijdschr. v. Geneesk. **1**: 1866, 1915.
150. Barbieri, D.: Diag. e tecn. di lab. **2**: 24, 1931.
151. Prus, J.: Medycyna, 1886, Nos. 39 and 40; Abst. Zentralbl. f. klin. Med. **8**: 469, 1887.
152. Casey, A. E., and Helmer, O. M.: Proc. Soc. Exper. Biol. & Med. **27**: 655, 1929, 1930; Ibid. **28**: 523, 1930, 1931.
153. Puchberger, G.: Wien. klin. Wchnschr. **18**: 711, 1905.
154. Howlett, E. H.: Lancet **1**: 223, 1882.
155. Afanassiew, M.: Deutsches Arch. f. klin Med. **35**: 217, 1884.
156. Maximow, A. A., and Bloom, W.: Textbook of Histology, Philadelphia and London, 1931, W. B. Saunders Co.
157. Schenk, P., and Spitz, S.: Med. Klin. **17**: 385, 1921.
158. Spitz, S.: Berl. klin. Wchnschr. **58**: 1073, 1921.
159. Nygaard, K. K.: Proc. Staff Meet. Mayo Clinic **8**: 365, 1933.

VALVES VERSUS THE MOTOR-BLOWER*

In the Benedict Type of Metabolism Apparatus Now in Common Use

Paul Roth, M.D., Battle Creek, Mich.

THE first metabolism apparatus of the Benedict type, the Universal,[1] was necessarily motor operated because of its very complex air circuit. It included four moisture and carbon dioxide absorption chambers besides many feet of tubing through which the air was forced by means of a Crowell rotary air pump.

The next Benedict type, the "portable"[2] or "Benedict-Collins,"[3] was considerably simplified and was adequately ventilated by means of a small electric air blower.

I presented in 1922[4] a modification, the "Benedict-Roth," in which the very simple "Sadd" valve was installed and the air blower discarded. The elimination of the latter was greeted with general favor and valves were soon universally adopted in all makes of the Benedict type of apparatus, including the "Benedict student form."[5]

All the electric air blowers installed in metabolism apparatus have the double action of a centrifugal pump: compression at the outlet and suction at the inlet. Depending on the location of the blower, the mouthpiece may be either in a zone of compression (a) or of suction (b). If in zone (a), inspiration will be assisted and expiration hindered, while in zone (b), inspiration will be hindered and expiration assisted. It is evident that breathing from

*Received for publication, May 21, 1934.

and into the current of air steadily maintained by an air blower cannot be favored during both phases of respiration. In fact, the greater the assistance during one phase, the greater will be the hindrance during the other. For physiologic reasons, interference with inspiration however slight is always objectionable and readily becomes distressing. On the other hand, resistance to expiration, if slight, is readily and totally ignored by the subject.

In a well-conditioned Benedict-Roth apparatus, with unobstructed connections and properly cared for soda lime, there is no appreciable resistance to air flow induced in the entire circuit by the act of breathing only. The best that can be done is to favor inspiration only to such a slight degree as to impose a correspondingly negligible resistance during expiration. In the writer's opinion, *there is no better way, to insure ease and comfort in breathing, than by simply having the spirometer bell slightly overbalanced, thus favoring inspiration.*

Extensive observations (1920) on oxygen consumption, pulse and respiration rates, with valves in comparison with the electric air impeller[4] proved that the latter was no longer necessary because of the elimination from the circuit of all but one absorber and much tubing, thus reducing air resistance to a minimum. (Incidentally, the resistance offered to the passage of the expired air through an ordinary charge of well-sifted soda lime, 4 to 8 mesh, is about the same as when passing through one foot of three-quarter-inch tubing. It is, in fact, negligible.)*

Recent substantial improvements, in compactness chiefly, insure still greater ease in breathing without the necessity of any troublesome mechanical contrivance of any kind.

Professor August Krogh, Copenhagen,[6] and the writer[4] published simultaneously (1922) but unknown to each other, their views in which both advocate the use of valves in the place of an electric blower.

On the other hand, a motor-blower of adequate efficiency is necessary when the apparatus is modified for allowing the use of a helmet as advocated by Benedict[7] or of such accessories as may impede the free circulation of air and impose any but negligible respiratory effort upon the subject.

The use of valves in the "Tissot" gasometer method has never been criticized. In fact, there is here no alternative, 100 per cent efficient valves are absolutely indispensable. The work imposed upon the subject in moving a well-compensated relatively heavy, 50 to 100 liter gasometer bell, has proved to be no appreciable impediment to breathing. There cannot, therefore, be any valid argument against the extremely negligible corresponding task in displacing the much smaller and lighter bell used in the "Benedict type" of apparatus.

In the twenty-two years during which in our service metabolism testing has been extensively carried on by means of every type of apparatus above referred to, our experience has been that a minimum of complaints is elicited when the quiet, valve apparatus is used.

Whether the valve or a motor-blower is used and no matter how carefully the test may be conducted patients will be encountered who dislike the ordeal

*Measurements made with a manometer inserted through the wall of the rubber mouthpiece of a properly conditioned valve apparatus showed a maximum water pressure of only 3 or 4 mm. during ordinary expiration and 8 to 10 mm. during forced expiration. No appreciable effect was noted during normal inspiration and only 5 or 6 mm. of negative pressure during forced expiration.

and may complain of difficult breathing or of something else. Tact and good technic will almost invariably take care of such subjects.

The use of the motor-blower rather tends to encourage neglect in the proper care of the apparatus and promotes in the mind of the operator a false sense of security even in the presence of more or less serious ventilation obstructions. In fact, too fine, too much, or impacted soda lime; too much resistance due to too long tubings; kinks or obstructions by accumulation of moisture; an improperly balanced spirometer bell, etc., may all be responsible for a partial or even a complete obstruction somewhere in the ventilation circuit. All this may take place during the test without any immediate apparent sign of trouble, while the motor is humming along as usual, while the spirometer bell is rising and falling without interference and while air continues to be available through the mouthpiece. In the absence of the valves, the air in the entire circuit can move in either direction and air will still be available through the mouthpiece in the presence of even a complete obstruction in the circuit. Under such conditions, more or less rebreathing of the expired air occurs and may be temporarily tolerated by the subject. The results of the test, however, should be questioned, chiefly on account of a possible deficiency in the oxygen supply.

There is an element of danger especially connected with the use of all air blower equipped metabolism and other respiration apparatus of the Closed Circuit type, including the various makes of oxygen therapy tents and artificial respirators.[8] I have previously called attention to the not altogether remote danger of acute anoxemia which is readily and insidiously induced by the rebreathing of air which is more or less completely freed from exhaled carbon dioxide. On account of the absence of an increased CO_2 concentration in the inspired air the body fails to make, for the time being, any discernable protest against an insidious and quite symptomless deficiency of oxygen until a more or less damaging degree of anoxemia or asphyxiation has been induced. Such an accident is practically impossible with a "valve" metabolism apparatus when the spirometer bell has been properly filled with oxygen before the test.

SUMMARY

The conclusions drawn twelve years ago can now be reiterated with greater emphasis:

1. The Benedict-Roth metabolism apparatus lends itself admirably to the adaptation of valves and the discard of an air blower.

2. The use of the apparatus is made available independently of any source of electrical energy.

3. The use of valves, together with a properly balanced spirometer bell, promotes easy and normal breathing and insures basal results.

4. The apparatus is absolutely noiseless and free from the possible transmission of disturbing impulses or "hums."

5. The valves seldom require attention. They will never have a tendency to stick if properly mounted and so adjusted that their "lips" just barely come together.

6. The positive, or negative, pressure maintained by the air blower throughout the entire circuit invites leaks, especially in the region of the mouth and the nose.

7. With the type of metabolism apparatus as now in common use, the electric impeller is unnecessary and sometimes a troublesome impediment.

8. The possibility of asphyxia which may be insidiously induced by the rebreathing of carbon dioxide freed air is not to be ignored, especially in the use of all motor operated metabolism, oxygen therapy, and artificial respiration apparatus.

REFERENCES

1. Benedict, F. G.: Am. J. Physiol. **24:** 345, 1909 also Deutsche Arch. f. klin. Med. **107:** 156, 1912.
2. Benedict, F. G.: Boston M. & S. J. **178:** 667, 1918.
3. Benedict, F. G.: Boston M. & S. J. **183:** 449, 1920.
4. Roth, Paul: Boston M. & S. J. **186:** 457, 491, 1922. Reprinted with Appendix: Bull. Battle Creek San. and Hosp. Clin. **18:** 98, 131, 1922.
5. Benedict, F. G.: Boston M. & S. J. **188:** 567, 1923.
6. Krogh, A.: Respiratory Apparatus for Clinical Determination of Energy Exchange in Man, Wien. klin. Wchnschr. **35:** 290, 1922. Published later in English in Boston M. & S. J. **189:** 313, 1923.
7. Benedict, F. G.: A Helmet for Use in Clinical Studies of Gaseous Metabolism, New England J. M. **203:** 150, 1930.
8. Roth, Paul: Oxygen Therapy, Current Researches in Anesthesia and Analgesia **8:** 47, 1929. Reprinted with Addenda in the Bull. Battle Creek Sanitarium & Hosp. Clinic **24:** 189, 1929.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

CHOLESTEROL, Relation of Plasma to Obesity and to Some of the Complicating Degenerative Diseases, Bruger, M., and Poindexter, C. A. Arch. Int. Med. 53: 423, 1934.

The plasma cholesterol in fifty-three obese subjects, in whom evidence of metabolic, arthritic or endocrine disturbances was lacking, was usually within normal limits.

The advent of diabetes mellitus, essential hypertension or arteriosclerosis in eighteen obese persons was associated in many instances with an augmented cholesterol content of the plasma. Four of seven overweight subjects who demonstrated a diminished tolerance to ingested carbohydrate as determined by the dextrose tolerance test, even though signs and symptoms of diabetes mellitus were lacking, showed an increased plasma cholesterol.

In four obese persons with infectious (rheumatoid or gonococcal) arthritis, the plasma cholesterol was usually within the normal limits; in seven cases with degenerative hypertrophic arthritis or osteoarthritis, the cholesterol was often significantly elevated.

From these observations it is concluded that:

A high caloric diet, presumably high in carbohydrates and fats, which results in obesity is not accompanied by a rise of the plasma cholesterol in human beings.

The development of degenerative diseases (hypertension, diabetes, arthritis and arteriosclerosis) in the obese is as a rule followed, and not preceded, by hypercholesteremia. Therefore, the elevated concentration of the cholesterol in the blood in these degenerative conditions is usually to be regarded as a complication and not in the light of an etiologic factor.

RHEUMATIC FEVER, Bacteriologic Study of Throats in Rheumatic and Non-Rheumatic Fever, Weinstein, I., and Styron, N. C. Arch. Int. Med. 53: 453, 1934.

The present investigation includes 321 cases and 840 cultures from Montefiore and Bellevue Hospitals, New York City. Forty-six per cent of the subjects were patients with rheumatic fever. Fifty-eight per cent of the cultures were taken from this group. The remainder were taken from normal persons and from patients suffering from diseases other than rheumatic fever; these constituted the control group. In more than one-third of the cases of rheumatic fever and in more than one-half of the cultures taken from this group the patients were under fifteen years of age.

In making cultures of hemolytic streptococci, it seems that rabbit blood is slightly better than horse blood. However, if appropriate dilutions are made, equally good results can be obtained with horse blood.

The percentage of cultures which were positive for the hemolytic streptococcus taken from the throats of patients with rheumatic fever was approximately the same as that found in other persons.

In these positive cultures, the hemolytic streptococcus appeared to be no more abundant in those from patients with rheumatic fever than in those from other persons.

Throat cultures of patients with rheumatic fever taken during an infection of the upper respiratory tract showed no greater incidence of hemolytic streptococci than those from other persons who were suffering from a cold or from sore throat.

Infections of the upper respiratory tract were found associated with *Streptococcus hemolyticus* more frequently in young persons who were suffering from rheumatic fever than in older patients with this disease.

Hemolytic streptococci were found more frequently associated with infection of the upper respiratory tract in rheumatic persons who had not had their tonsils removed than in those on whom tonsillectomy had been performed.

Green-producing streptococci were present in all the cases and in 99 per cent of the cultures. They predominated in 69 per cent of the cases and in 73 per cent of the cultures.

Indifferent streptococci were present in 96 per cent of the cases and in 88 per cent of the cultures. They predominated in 3 per cent of the cultures.

In the groups studied, exacerbations of acute rheumatic fever occurred as frequently when there was no infection of the throat as when there was.

The fact that the majority of those in whom exacerbations of rheumatic fever occurred were persons who had hemolytic streptococci in their throats suggests a possible relationship between this organism and the reappearance of the symptoms.

TETANUS, Acute, Study of the Treatment of, Taylor, F. W. J. A. M. A. 102: 895, 1934.

1. *Local Lesion:* Since the local wound causes the disease and continues it, prime consideration should be given to that focus. Whenever possible, immediate complete excision of the wound is recommended. When this is not possible, complete exposure and search for foreign bodies should be done under general anesthesia. The foreign body is a potent factor aiding bacterial growth and continuing the elaboration of toxin. It should not be overlooked. In active tetanus cases it must be considered that the local wound contains a foreign body until proved otherwise. This is particularly true of "blank" cartridge wounds. The local wound of tetanus is an acute surgical emergency and should be treated as such, even though the patient is later admitted to the medical wards.

2. *Sedatives and General Care:* Patients with tetanus die from "their symptoms and not from the disease itself." It therefore follows that if these symptoms can be controlled adequately the patient's chance of recovery is much greater. Tribromethanol or sodium amytal is recommended to induce light narcosis. The suggestion is also made that these drugs be given at regular intervals so as to keep the patient quiet and relaxed. This would seem wiser than to use them only when the patient becomes rigid and is on the verge of a convulsion. Equally important in conserving the patient's strength with sedatives is adequate supportive care. Particular attention must be given so that fluid intake is maintained at a high level and proper elimination is effected.

3. *Tetanus Antitoxic Serum:* The high reputation that this product enjoys is due entirely to its prophylactic use. Here it is a specific in preventing the disease or at least in lengthening the incubation period. In active tetanus it is in no sense a specific, and its value is questioned by many. As the antitoxin is unable to withdraw toxin already combined with nervous tissue, its sole action is to neutralize uncombined toxin in the body. Here it may have considerable value and, until definite proof to the contrary, its use may well be continued. It seems unnecessary to administer the huge quantities used at present. It is recommended that from 30,000 to 60,000 units be given when the patient is first seen. This might be repeated if the case runs a long, protracted course. The intramuscular route of injection is perhaps the most satisfactory.

The conclusion of two workers in this field nearly forty years ago is as pertinent today as it was then: "An ounce of clean surgery is worth several pounds of serum therapy."

SYPHILIS AND PREGNANCY, Mc Kelvey, J. L., and Turner, T. B. J. A. M. A. 102: 503, 1934.

The Wassermann test on the blood of the umbilical cord and study of the placental histology are important aids in the diagnosis of congenital syphilis and should be carried out on all patients not proved during pregnancy to be free from syphilis. Of the two, the former is the more reliable.

The presence of characteristic changes in the epiphyses of the long bones during the first two weeks of life is diagnostic of congenital syphilis, but the absence of epiphyseal abnormalities does not rule out congenital infection.

Antenatal treatment of pregnant syphilitic patients with arsphenamine reduces the percentage of fetal deaths and the percentage of syphilitic infants in a striking manner. The good results are roughly proportional to the amount of treatment given and the time at which it is started; even a few treatments in the last weeks of pregnancy, however, will materially alter the outcome.

IMPETIGO CONTAGIOSA, Prophylaxis of, Guy, W. H., and Jacob, F. M. J. A. M. A. 102: 840, 1934.

The following method, used since 1930, has been found efficacious.

A. Delivery Room.—

1. As soon as possible after birth, babies are cleansed thoroughly with sterile liquid petrolatum.
2. Each baby is thoroughly rubbed from the top of the head to the soles of the feet with 2 per cent ointment of ammoniated mercury before it leaves the delivery room.

B. Nursery.—

1. Daily cleansing is accomplished with sterile cottonseed oil.
2. The buttocks and anus are cleansed with cotton balls and cottonseed oil.
3. Soap and water and powder are not used.

HYPOCALCEMIA AND MIGRAINE, Norman, G. F. J. A. M. A. 102: 529, 1934.

A number of cases of typical so-called idiopathic migraine have been studied with respect to blood calcium values, which have been found to be uniformly somewhat depressed. Six cases have been chosen at random, ranging from relatively mild to very severe, the last being associated with epileptiform convulsions. This entire group has been benefited by means taken to raise the values of calcium to normal.

It is assumed that symptoms of migraine and tetany are maintained dormant in this group by the calcium regulating mechanism.

One or the other complex may predominate. Whether unilateral headache and nausea with or without vomiting should be included in the picture recognized as tetany remains for further study.

MERCURY POISONING, An Antidote for, Rosenthal, S. J. A. M. A. 102: 1273, 1934.

Sodium formaldehyde sulphoxylate saved nine of twelve dogs from a fatal oral dose of corrosive mercuric chloride, when administered by mouth and intravenously within an hour and a half after the poison had been taken. The nine surviving animals were protected against kidney damage, as shown by the lack of elevation of the blood nonprotein nitrogen. In the dogs that succumbed following this therapy or following intravenous therapy only, no significant renal lesions were demonstrable histologically.

The sulphoxylate was used in ten human cases of acute poisoning from corrosive mercuric chloride, and recovery occurred without appreciable kidney damage.

SPINAL FLUID, Cell Counts in Epidemic Encephalitis and Tuberculous Meningitis, Guerrero, L., and Ignacio, P. J. Philippine Islands M. A. 14: 81, 1934.

The total cellular count of the spinal fluids in adult encephalitis cases, numbering 49 (44 recoveries and 5 deaths) varied from 1 to 3,067 with an average of 139. As it is unusual to find epidemic encephalitis with total cell counts amounting to several hundred or thousand cells per cubic millimeter, it is possible that these cases might have been cases of so-called lymphocytic meningitis, the exact cause of which is not very well known.

The total cell counts in 20 children with encephalitis (13 recoveries and 7 deaths) varied from 1 to 110, with an average of 23 cells per cubic millimeter.

In tuberculous meningitis (34 adults and 98 children) the average total cell counts were higher than those for encephalitis in both adults and children.

The average polynuclears and lymphocytes were practically the same in both diseases, but there was always a marked preponderance of lymphocytes over polynuclears.

No definite relation was found between the total cell count in and the severity of encephalitis, even during the different periods of the disease.

As all the tuberculous meningitis cases in our series died, the relation mentioned in the preceding statement could not be determined.

Normal cell counts were found in cases of encephalitis and tuberculous meningitis, and the probable explanations are given.

In a few cases of encephalitis and tuberculous meningitis, especially at the beginning, predominance of polynuclears was observed, which may cause it to be confused with suppurative meningitis; but confusion can be definitely eliminated by further examination of the spinal fluid at different periods.

Finally, in the author's opinion, one cannot rely alone on the cellular counts in the spinal fluid in differentiating between encephalitis and tuberculous meningitis; therefore, we must look for other reliable and quick methods, either in the clinics or in the laboratory.

KALA-AZAR, New Diagnostic Test for, Nattan-Larrier, L., and Grimard-Richard, L. C. R. Soc. Biol. **113**: 1489, 1934.

The authors describe a new serological test for leishmania infections based on the fact that alexine is adsorbed by a mixture of the serum from a case of leishmania infection and the serum of a rabbit which has been immunized with cultures of *Leishmania donovani*. The rabbit serum is prepared by injecting a rabbit intravenously with a culture every six days until 6 injections have been given. The blood is abstracted 15 days after the last injection, defibrinated and centrifuged, the resulting serum being placed in ampules which are kept in the ice chest. The test is carried out in two ways. In the first 0.1 c.c. of alexine, 0.1 c.c. of the suspected inactivated leishmania serum, decreasing quantities (one-tenth to one hundred millionth of 1 c.c.) of the rabbit serum (inactivated if fresh) and 0.2 c.c. of physiological saline solution are mixed in tubes and kept at a temperature of 37° C. for one and one-half hours. To each tube are then added 0.1 c.c. of antisheep serum and 0.1 c.c. of 5 per cent suspension of sheep corpuscles. The tubes are then kept at 37° C. for one-half hour. If the suspected serum was actually from a case of leishmania infection hemolysis does not occur owing to fixation of the alexine. In the second way of making the test the suspected serum is present in decreasing quantities, the other ingredients being uniform.

The two methods give good results but the second is preferable if small quantities only of suspected serum are available. Positive results have been obtained with one human and six canine cases of kala-azar and with mice suffering from *Leishmania tropica* infection of the tail.

TYPHOID BACILLUS, A Study of "Smooth" and "Rough" Forms of, In Relation to Prophylactic Vaccination and Immunity in Typhoid Fever, Maltaner, F. J. Immunol. 26: 161, 1934.

Methods of dissociation were applied to a group of strains of the typhoid bacillus, including recently isolated cultures from "carriers" and several well-known laboratory strains.

Typical smooth and rough variants were obtained from both flagellated and nonflagellated forms. Capsules were demonstrated on all forms: rough, smooth, flagellated, and nonflagellated.

No serologic relationship was observed in agglutination and agglutinin-absorption experiments between the somatic antigens of smooth and rough races of *B. typhosus* although the existence of a common flagellar antigen was confirmed.

Living suspensions of all flagellated and of some nonflagellated rough forms failed to be agglutinated by pure rough somatic antiserum. They did agglutinate, however, when the suspensions were heated to a temperature which destroys flagella and probably also capsules.

By intravenous inoculation of rabbits with suspensions of living rough and smooth variants, the relative invasive and toxic character of these forms was determined. No differences in toxicity were observed by this method. Only the smooth variants, however, were found to possess invasive properties as determined by development of the carrier state.

Vaccination of rabbits by the intravenous method with adequate dosage of either rough or smooth variants gave protection against approximately fourteen times the minimal invasive dose of smooth living bacilli. The rough vaccines protected distinctly better than the smooth. The sera of rabbits vaccinated with nonflagellated rough vaccines did not possess specific agglutinins for the smooth infecting strain. Those vaccinated with rough flagellated forms possessed only flagellar agglutinins.

The inoculation of normal rabbits intravenously with rough variants of the typhoid bacillus resulted in a more marked and persistent leucocytosis than when smooth bacilli were used. Immunized rabbits, regardless of whether rough or smooth vaccines had been used, responded after the usual primary leucopenia with a hyperleucocytosis upon intravenous injection of smooth living cultures, whereas the response of unvaccinated rabbits to the injection of virulent smooth bacilli was relatively slight.

The intraperitoneal inoculation of living rough and smooth typhoid bacilli into guinea pigs indicated that the smooth variant was more toxic than the rough. However, the inoculated smooth bacilli multiplied very rapidly in the peritoneum whereas similar doses of the rough variant were disposed of by prompt phagocytosis.

The rough variant, as indicated, is phagocyted more vigorously than the smooth in the peritoneal cavity of the guinea pig—both within the peritoneal exudate and on and within the omentum and liver. This phagocytic response to rough organisms in normal guinea pigs parallels the reaction obtained when smooth bacilli are inoculated into the peritoneal cavity of guinea pigs immunized with either rough or smooth vaccines.

The results of this experimental investigation are, in every particular, in conformity with the practical experience of the past thirty years in preventive inoculation of human subjects against typhoid fever.

DYSENTERY BACILLI, Classification of, Shiga, K. Zentralbl. Bakt. 130: 1, 1934.

Shiga proposes that the dysentery bacilli be divided into the following groups: (1) dysentery, (2) metadysentery, and (3) paradysentery.

The differentiation is shown in the table:

ACCORDING TO LENTZ	1 SHIGA-KRUSE BACILLUS	2 SCHMITZ BACILLUS	3 FLEXNER GROUP			4 KRUSE-SONNE BACILLUS
			FLEXNER	Y	STRONG	
Litmus mannite	blue	blue	red	red	red	red
Indole	−	+	+	±	±	+
Milk	no coagulation	no coagulation	no coagulation			coagulation
Litmus lactose	blue	blue	blue			red
Special toxin	+	−	−	−	−	−
According to Shiga	(1) *B. dysenteriae*	(2) *Bacillus metadysenteriae*				(3) *B. paradysenteriae*

DIPHTHERIA TOXOID, Immunity Produced by, Greengard, J. Am. J. Dis. Child. 47: 799, 1934.

In a series of 214 susceptible infants ranging from newborn to two years of age, 93.4 per cent showed a negative reaction to the Schick test after two injections of 1 c.c. of commercial diphtheria toxoid.

Of 63 immunized infants retested at intervals after the first negative reaction to the Schick test, 11 or 17.2 per cent reverted to a positive reaction.

Of the 14 patients who failed to demonstrate a negative reaction to the Schick test after vaccination, 13 were immunized during the first half year of life. Of the 11 immunized infants whose reactions reverted to positive, 10 were immunized when under six months of age.

In a small group of infants in whom the duration of immunity could be tested, it was noted that the immunity seemed more lasting in the infants who demonstrated immunity to the Schick test most rapidly.

There is a definite relationship between the antigenic power of the immunizing agent used and the results of vaccination.

Immunization should never be considered complete without a follow-up Schick test.

ANTIGEN, Method of Diluting, in Relation to Wassermann Reaction, Kolmer, J. A., and Richter, C. E. Am. J. Clin. Path. 4: 301, 1934.

The manner or method of diluting extract for the Wassermann test has a slight but definite influence upon antigenic sensitiveness.

Turbid emulsions of antigens secured by slow or gradual dilution with saline solution are more antigenic than opalescent emulsions prepared by rapid dilution.

Turbid emulsions of antigens prepared by adding extract drop by drop to saline solution, with constant shaking, are more antigenic than turbid emulsions prepared by adding small amounts of saline solution to antigen with constant shaking.

There is no detectable influence in the manner of diluting Kolmer antigen upon the hemolytic properties of this extract.

Turbid emulsions of antigens prepared by slow dilution are sometimes slightly more anticomplementary than opalescent emulsions prepared by rapid dilution.

In the Kolmer modification of the Wassermann test, it is recommended that the antigen be diluted by adding it drop by drop to the required amount of saline solution with constant shaking to secure the maximum of turbidity as originally described in this method.

"CARRIERS," Practical Limitations in the Attempt to Control Enteric Disease by the Examination of Specimens Collected Without Regard to Clinical History or Epidemiological Evidence, Gilbert, R., and Coleman, M. B. Am. J. Pub. Health 24: 1, 1934.

A false sense of security may result from the examination of a specimen or two submitted from each food handler, since every carrier of *B. typhosus* would not be discovered by this procedure. Even when a number of plates are used in the study and a most careful search is made, *B. typhosus* may be found in only a relatively small percentage of the fecal specimens obtained from some typhoid carriers.

The study of environmental factors, careful history-taking, and the submission of a series of specimens of feces and urine, or preferably a specimen of duodenal contents from food handlers who have had enteric disease, colitis, cholecystitis, etc., or whose immediate associates have had typhoid or paratyphoid fever, would probably result in the detection of most carriers of typhoid or paratyphoid bacilli among them. Of course, there are exceptions. When cases of enteric disease have occurred among patrons of a restaurant or on a milk route, series of specimens from all the food handlers concerned should be examined if the carrier is not found among those with suggestive histories.

Despite the fact that unrecognized cases occur, probably one of the best means of approaching the problem is by studying specimens from patients who are convalescing from typhoid fever or allied infections, in order to detect the ones who will become carriers. Since the submission of specimens from persons who have recovered from typhoid fever has been required by the New York State Sanitary Code, no cases of the disease, as far as the authors are aware, have been traced to an individual who has been released after fulfillment of the requirements.

In order to avoid the fruitless expenditure of time and materials in the examination of large numbers of specimens that have been collected without discrimination from various groups of individuals, the relative futility and excessive cost of such work should be ex-

plained to members of county medical societies and health officials. The same amount of money would yield vastly greater returns if expended in the pasteurization of products wherever possible, the improvement of sanitary facilities, the provision of training in personal hygiene, the removal from work and the treatment of food handlers who are obviously ill, the careful study of epidemiological factors, and the examination of series of specimens from individuals whose history or clinical manifestations warrant it.

PSITTACOSIS, Pathology of, Foord, A. G. Am. J. Clin. Path. 4: 247, 1934.

The principal finding in fatal human cases of psittacosis is a unique pneumonia, involving diffusely the greater part of all of one or more lobes, producing a wet, consolidated parenchyma which is smooth and nongranular on section. The pleura, bronchi, and bronchioles are relatively spared. Histologically the consolidation is found to be due to an exudate consisting of fluid and varying amounts of fibrin, in the meshes of which large numbers of large mononuclear cells are found. Proliferation of the alveolar epithelium and histiocytes in the alveolar walls is a constant finding.

Moderate splenic tumor and congestion and cloudy swelling of the parenchymatous viscera is the rule.

Edema and congestion of the brain and leptomeninges is constant, ring-shaped hemorrhages in the cord and brain have been found in some cases, but thorough neurologic study should be made on all cases, since delirium and psychosis are clinically found in the majority of cases.

Postmortem diagnosis should be confirmed by inoculating white mice with material from the lung, liver, and spleen. Ante mortem diagnosis is usually not possible before the patient recovers or dies but can sometimes be made by inoculation of a series of white mice intraperitoneally with the patient's blood, or better with the sputum.

ARTHRITIS, Chronic Nonspecific, Archer, B. H. J. A. M. A. 102: 1449, 1934.

There appears to be a basis for the concept that both rheumatoid arthritis and osteoarthritis are due to the same etiologic agent or group of agents and that the proliferative and degenerative pathologic changes by which the two types manifest themselves are the result of other factors than those of causation.

There seems to be no conclusive evidence of the presence of streptococci in the blood and joints of patients with chronic arthritis.

None of the vaccines employed at the present time in the treatment of chronic arthritis have been accepted by the Council on Pharmacy and Chemistry. There is no evidence at hand that they exercise any specific effect on the course of the disease.

Dietary regulations and vitamin therapy apparently exercise no specific effect on the joint manifestations of patients with this disease.

In those cases associated with foci of infection it seems wise to search for and remove this factor early in the course of the disease.

In advanced cases the measures the author has found to be of greatest benefit to the patient are orthopedic procedures, physical therapy, the administration of drugs to allay pain, and a change of climate.

Present knowledge of the subject does not seem to warrant the view that certain definite measures should be applied only to certain definite types of arthritis. There is no conclusive evidence that the same measures do not apply at some time to all forms of nonspecific arthritis.

The Journal of Laboratory and Clinical Medicine

VOL. 20 JANUARY, 1935 NO. 4

Editor: WARREN T. VAUGHAN, M.D.
Richmond, Va.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - - - - - - CINCINNATI
J. J. R. MACLEOD, M.B. - - - - - - ABERDEEN, SCOTLAND
W. C. MACCARTY, M.D. - - - - - - - ROCHESTER, MINN.
GERALD B. WEBB, M.D. - - - - - - - COLORADO SPRINGS
VICTOR C. MYERS, PH.D. - - - - - - - - - CLEVELAND
RUSSELL L. HADEN, M.D. - - - - - - - - CLEVELAND
JOHN A. KOLMER, M.D. - - - - - - - - PHILADELPHIA
ROBERT A. KILDUFFE, M.D. - - - - - ATLANTIC CITY, N. J.
GEORGE HERRMANN, M.D. - - - - - - - - GALVESTON
T. B. MAGATH, M.D. - - - - - - - - ROCHESTER, MINN.
DEAN LEWIS, M.D. - - - - - - - - - - BALTIMORE
M. H. SOULE, SC.D. - - - - - - - - ANN ARBOR, MICH.
CLIFFORD J. BARBORKA, M.D. - - - - - - - - CHICAGO

Contents of this Journal Copyright, 1935, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second Class Matter

EDITORIAL

The Problem of Peptic Ulcer

IN LAST analysis, the etiology of peptic ulcer still remains an unsolved problem. It seems probable that of the many etiologic factors that have been suggested in the past, several or all may play a part at times, but no one explanation or group of explanations so far proposed seems to present a complete solution in all cases. Metastasis from foci of infection, corrosive action of hydrochloric acid, enteric excretion of toxic substances, trauma, and excess heat may be factors of importance in individual cases but there is also evidence of a fundamental predisposition, in the absence of which these factors remain inactive.

The problem of trauma is indeed interesting. One of the difficulties of experimental investigations on the etiology of ulcer appears to be the great difficulty in producing chronic ulcer in animals. Traumatic ecchymoses may be produced in the stomach wall hematomas, and indeed these may become infected, resulting in acute ulceration. But such an experimentally produced

ulcer remains an acute ulcer which heals spontaneously. Experimental methods have been notably unsuccessful in producing chronic peptic ulcer.

There is evidence that the same situation holds in man. Direct trauma may produce an acute ulcer but even in man such an ulcer usually passes on to spontaneous cure. Crohn and Derendasy[1] have reviewed the literature on traumatic ulcer of the stomach and duodenum. Apparently acute traumatic ulcer is recognized more frequently on the European continent than in this country. These authors suggest that if the possibility of this condition be borne in mind, it may be that it will be recognized more frequently. Its medicolegal importance is obvious.

The first case of traumatic ulcer to appear in the literature was described by Potain in 1856. Although this case progressed into chronic ulceration which persisted until death, the fact remains that the majority are evanescent. With many of those cases in which the symptoms persist one cannot be certain that there was no preexisting ulcer before the abdominal trauma.

Gray[2] reports five cases of ulcer following external trauma. In one the ulcer appears to have been chronic, but here again the complete absence of ulcer prior to the trauma was not established.

We are brought back to a consideration of the possibility of a diathetic predisposition as an explanation for the fact that upward of 5 per cent of the population experience gastric or duodenal mucosal ulceration at one time or another, while others of the population in spite of grossest insults to their digestive apparatus fail to develop the malady.

The possibility of an allergic relationship has recently been brought up. Rowe[3] first pointed out that food allergy may produce symptoms indistinguishable from those of ulcer. Kern and Stewart[4] have discussed allergy in duodenal ulcer. They found that individuals with duodenal ulcer who happened to be allergic to milk were not relieved from their ulcer symptoms until after this specific allergenic food had been eliminated from their diet. Thereafter relief was rapid.

Recently L. P. Gay[5] of St. Louis has reported allergic studies of a series of cases with peptic ulcer. He has found a number sensitive to one or another food, other than milk, and has achieved relief in his series following specific food avoidance, in cases in which, prior to the establishment of allergic restrictions, there had been no relief.

Bearing in mind that the characteristic allergic response is that of smooth muscle spasm, possibly with a resulting local ischemia, thereby predisposing to exaggerated response to nonspecific factors such as trauma, infection, corrosive action and the like, one can conceive a localized allergic reaction as being a factor. Although there is a justified skepticism regarding the tendency to explain all things otherwise unexplainable on the grounds of allergy, at the same time the number of manifestations in which an allergic factor has been quite conclusively demonstrated is truly surprising. And the list is gradually growing.

It is not our purpose to take sides as to whether allergy may be a predisposing or exciting factor in the development of ulcer. This cannot be determined by forensic methods but must await the appearance of confirma-

tory studies and additional clinical and experimental work. It has already been demonstrated conclusively that local infection or irritation will determine a concentration of reinjected antigen in the irritated or infected tissues. Thus it might be interpreted that allergy may be playing an exciting rather than a predisposing rôle, when it does play a part in ulcer.

The interesting point of the studies herein reviewed is that we are coming increasingly to a realization that further advances in our understanding of the etiology of peptic ulcer will depend in great measure upon increased knowledge of the, as yet, poorly understood factor of tissue predisposition.

REFERENCES

1. Crohn, Burrill, and Derendasy, Julius: Traumatic Ulcer of the Duodenum and Stomach, J. A. M. A. **100**: 1653, 1933.
2. Gray, I.: Relation of Peptic Ulcer to External Trauma, Ann. Int. Med. **7**: 1403, 1934.
3. Rowe, Albert H.: Food Allergy, Its Manifestations, Diagnosis and Treatment, J. A. M. A. **91**: 1623, 1928.
4. Kern, R. A., and Stewart, S. T.: Allergy in Duodenal Ulcer: Incidence and Significance of Food Hypersensitiveness as Observed in 32 Patients, J. Allergy **3**: 51, 1931.
5. Gay, L. P.: Southern M. J. In press.

—*W. T. V.*

CORRESPONDENCE

To the Editor:

Dear Sir: The purpose of this communication is to call attention to the fact that we have been misquoted in the article by L. F. Herz (JOURNAL OF LABORATORY AND CLINICAL MEDICINE 20: 33, 1934), in which he states on page 39, "Since this article was written Kracke and Parker have reported several cases following the use of 'empirin,' a mixture of phenacetine, aspirin and caffein." First, we did not report several cases following the use of this mixture, and second, empirin is not a mixture of phenacetine, aspirin, and caffein; but only a trade name for acetylsalicylic acid. Further comment relative to this article seems indicated.

First, it is evident that the author has attempted to show that the leucocyte depressing effect of amidopyrine is due to its pyrazalon attachment. We do not believe that he establishes this. He further states, "I am prepared to show that amidopyrine should not be classed as a benzene ring derivative." We do not believe that he has demonstrated this. After close examination of the chemical reactions presented by the author we note many errors; for example, McGuigan states that amidopyrine is a derivative of phenylhydrazine that comes ultimately from aniline and benzene and is stated by that author to be a benzene derivative. It is not prepared from pyrazalon.

It is stated on page 36, that amidopyrine is more toxic than acetanilid and phenacetine, not because of the pyrrol ring, but because of the NH component of the ring. This NH or N is called, on page 37, an imido radical and to this fact is assigned its toxicity. It must be pointed out that this structure is not an imido radical but an amine radical and occurs, not only in amidopyrine but also in acetanilid and phenacetine. An imido radical must be attached to two acyl groups, otherwise it is an amine as in the case of amidopyrine.

On page 37, is shown by diagram, several drugs which are said to have the NH or N group as an integral part of the ring. In this group are listed both anilin and phenyl hydrazine, neither of which has these groups in the ring. Instead they are merely side chains attached to the ring.

On the same page it is stated that piperidine differs from pyridine only in the substitution of an NH radical for the N radical. This is in error since pyridine has a constitutional formula of C_5H_5N while piperidine has the formula $C_5H_{11}N$.

It is also stated, page 37, that phenyl hydrazine contains the imido radical, which is in error as this NH is an amine.

Since the author presents little evidence to show that amidopyrine is not a benzene ring derivative, it follows that some of his conclusions are unwarranted. Although there is much evidence to indicate that amidopyrine is a dangerous leukocyte depressing drug only in an occasional person; its dangerous character has not been so well established that it should be banned by the medical profession. Amidopyrine is a very valuable drug when properly used and in all probability it should continue to be used with caution just as we continue to use neoarsphenamine, although we recognize its ill effects in an occasional person.

The author goes beyond the scope of his article in drawing conclusion number six when he states that acetanilid is the drug of choice when anodynes or antipyretics are indicated. And still further unwarranted is the conclusion that it should be mixed with caffeine and potassium citrate, since there is no scientific evidence to show the desirability of using such a mixture in therapeutics.

ROY R. KRACKE, M.D.,
AND
FRANCIS P. PARKER, M.D.

NOVEMBER 5, 1934,
EMORY UNIVERSITY, GA.